PREDICTORS, OUTCOME, PROFILE OF ANTI-TUBERCULAR DRUG INDUCED HEPATITIS

A prospective nested case- control study in a South Indian tertiary hospital

Dr.Selvin Sundar Raj M

PREDICTORS, OUTCOME, PROFILE OF ANTI-TUBERCULAR DRUG INDUCED HEPATITIS

– A prospective nested case- control study in a South Indian tertiary hospital

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE M.D. BRANCH I (GENERAL MEDICINE) EXAMINATION OF THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI TO BE HELD IN APRIL 2015.

DECLARATION

This is to declare that this dissertation titled**"PREDICTORS, OUTCOME, PROFILE OF ANTI-TUBERCULAR DRUG INDUCED HEPATITIS – A prospective nested case-control study in a South Indian tertiary hospital"** is my original work done in partial fulfilment of rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai to be held in April 2015.

CANDIDATE

Dr.Selvin Sundar Raj.M

Post graduate Registrar

General Medicine

Christian Medical College

Vellore

CERTIFICATE

This is to certify that the dissertation entitled "**PREDICTORS, OUTCOME, PROFILE OF ANTI-TUBERCULOSIS DRUG INDUCED HEPATITIS – A prospective nested case- control study in a South Indian tertiary hospital**" is the bonafide original work of Dr. Selvin Sundar Raj.M, in fulfillment of the rules and regulations for the M.D. Branch-I (General Medicine) Degree Examination of the Tamil Nadu Dr. M.G.R University, Chennai, to be conducted in April 2015.

Guides Signature:

Dr. Ramya.I
Associate Professor & Head of Unit,
Department of Medicine Unit V,
Christian Medical College and
Hospital,
Vellore – 632002.
Tamil Nadu.

Dr. Anand Zachariah,
Professor & Head,
Department of General Medicine,
Christian Medical College and
Hospital,
Vellore – 632002.
Tamil Nadu.

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PRINCIPAL

Dr.Alfred Job Daniel
Professor
Dept of Orthopaedics
Christian Medical College
Vellore

HEAD OF THE DEPARTMENT

Dr.Anand Zachariah
Professor and Head
Department of Medicine
Christian Medical College
Vellore

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TURNITIN ORIGINALITY REPORT

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Contents

ABSTRACT

TITLE OF THE ABSTRACT:

PREDICTORS, OUTCOME, PROFILE OF ANTI-TUBERCULAR DRUG INDUCED HEPATITIS – A prospective nested case - control study in a South Indian tertiary hospital.

NAME OF THE CANDIDATE : Selvin Sundar Raj. M

DEPARTMENT : General Medicine

NAME OF GUIDES : Dr. Anand Zachariah and Dr. Ramya. I

DEGREE AND SUBJECT : M.D., Branch I, General Medicine

KEY WORDS: Anti-tubercular drugs, hepatitis, DOTS and daily regimen, rechallenge

OBJECTIVES:

This study was conducted to determine the incidence of anti-tubercular drug (ATT) induced hepatitis and to study the clinical risk factors, clinical, laboratory profile and outcome of patients with ATT induced hepatitis.

METHODOLOGY:

This case-control study was nested in a cohort of patients from Christian Medical College, Vellore. It was carried out from April 2014 to May 2015. All patients newly diagnosed to have tuberculosis, started on ATT were eligible for this study. All patients who present with suspected ATT related hepatotoxicity were also enrolled in the study. All patients were clinically assessed for symptoms of hepatitis at every visit until completion of treatment. Once the patient

developed ATT induced hepatitis, all hepatotoxic drugs were stopped and a non-hepatotoxic regimen was continued. Once the liver function tests normalized, patients were re- introduced with first line regimen as per decision of the treating physician and followed up till the completion of treatment. The incidence of ATT induced hepatitis was obtained from the cohort. The identification of risk factors of ATT induced hepatitis was based on a case control design nested in the cohort study. A descriptive study of clinical profile and outcome of patients with ATT induced hepatitis was also conducted. The risk factors for ATT induced hepatitis were identified by bivariate analysis and logistic regression analysis with odds ratio and 95 % confidence interval.

RESULTS:

A total of 393 patients were eligible for our study which included 5 patients presenting with ATT induced hepatitis. In the cohort, 61% were male and 81% were in the age group 20-59. HIV infection was found in 72 patients (18.3%). One hundred and fourteen patients (29%) were started on DOTS regimen and the remaining 279 patients (71%) were treated with weight based daily regimen. Patients on DOTS regimen had lower rates of HIV infection and disseminated disease but had greater undernutrition when compared with patients on daily regimen. Majority of the patients (38.9 %) patients had sputum positive pulmonary tuberculosis. A total of 281 patients (72%) had localized disease and 112 patients (28%) had disseminated disease. Forty three patients out of 393 patients developed DILI. The incidence of anti-tubercular drug induced liver injury was 9.7 % (95% C.I 7-13.2%) with lower incidence among patients on DOTS regimen (DOTS 3.5% (95% C.I 2.4%-4.8%) Vs Daily 14% (95% C.I 7.9 – 22.4%)).

HIV infection (OR 2.84, p value 0.002, 95% C.I 1.42 – 5.67), daily regimen (OR 4.46, p value 0.003, 95% C.I 1.55 – 12.81), disseminated disease (OR 1.769, p value 0.006, 95% C.I 1.23-2.55), hypoalbuminemia (OR 1.92, p value 0.045, 95% C.I 1.01 – 3.68) and chronic liver disease (OR 4.72, p value 0.004, 95% C.I 1.5-14.82) were independent risk factors for development of drug induced liver injury. On multivariate logistic regression analysis, HIV infection, hypoalbuminemia, chronic liver disease and daily regimen were found to be significant risk factors for DILI. A prediction score based on the above risk factors is suggested to identify patients who will develop DILI. A score of $\geq$ 5 will predict DILI with a sensitivity and specificity of 74% and 67%.

Vomiting was the most common symptom seen in 58.1% of patients with drug induced hepatitis followed by jaundice in 30.2 % of patients. Four patients developed acute liver failure. The majority of patients (77%) developed drug induced liver injury within first 2 months. The mean time duration for normalization of liver function was 22 days ranging from 3 to 81 days. Fifteen patients (35%) had severe hepatitis. All cause mortality in DILI was 4.7 % (2 patients). 36 patients (84%) had complete resolution of hepatitis. At least 1 drug was successfully rechallenged in 28 out of 29 patients. Rechallenge by both ATS and BTS guidelines had similar successful rechallenge. The rates of rechallenge hepatitis were similar in patients who were rechallenged according to both ATS and BTS guidelines (13.3% Vs 13% respectively).

CONCLUSION:

Incidence of ATT induced hepatitis from our study was 9.7% (95% C.I 7-13.2%) with lower incidence among patients on DOTS regimen. HIV infection, daily regimen, disseminated disease, hypoalbuminemia and chronic liver disease were independent risk factors for

development of DILI. Mortality rate was low (4.3%) among patients who developed DILI. Rechallenge by both ATS and BTS guidelines had similar successful rate. The predictive scoring system proposed from our study needs to be validated by a well designed prospective study. The study suggests that the combination of risk factors of extensive TB disease, HIV and undernutrition increase the vulnerability to drug induced liver disease particularly with daily TB treatment regimen, emphasizing the role of acquired risk factors in the development of DILI.

Aim

- To study the incidence and risk factors of anti-tubercular drugs induced hepatitis.
- To study the profile and outcome of patients with ATT induced hepatitis.

Objectives

- To determine the incidence of hepatitis among patients on anti-tubercular drugs.
- To study the clinical risk factors for ATT induced hepatitis.
- To study the clinical and laboratory profile of the patients with ATT induced hepatitis.
- To study the outcome of patients with ATT induced hepatitis.

Review of literature

Introduction

Tuberculosis (TB) remains a major global health problem. It ranks as the second leading cause of death worldwide from an infectious disease, after the human immunodeficiency virus (HIV). In India, there were nearly 2.2 million new cases of tuberculosis in 2011 and 300,000 TB related deaths in India (2). This is despite the availability of treatment that will cure most cases of TB. The first line drugs used for the treatment of tuberculosis include Isoniazid, Rifampicin and Pyrainamide all of which are hepatotoxic. Incidence of Anti- tuberculosis Drug induced Hepatitis varies from 3 -28% in various studies with higher incidence in Asian countries (1). The reason why some people develop hepato-toxicity despite all patients receiving the same doses of drugs is unclear. Several clinical risk factors have been identified including age, female sex, abnormal baseline liver function test (LFT), malnutrition, underlying liver disease, HIV infection and extent of tuberculosis. Also genetic susceptibility of the patients to drug liver injury has been identified such as NAT2 polymorphism. Despite this knowledge we are not yet able to predict development of ATT induced hepatitis before initiation of treatment. Hence we aim to study the incidence and risk factors of ATT induced hepatotoxicity so that in future, patients at risk can be identified early and serious hepatic complications and death prevented.

Epidemiology

Geographically, the burden of TB is highest in Asia and Africa. India and China together account for almost 40% of the world's TB cases(2). The most effective anti – tuberculosis drugs comprises of Isoniazid, Rifampicin and Pyrazinamide all of which are hepatotoxic. ATT induced hepatitis can lead to treatment failure and further contribute to Multi Drug Resistant (MDR) tuberculosis as a result of sub-optimal TB treatment regimens. Worldwide, 3.7% of new cases and 20% of previously treated cases were estimated to have MDR-TB (2).

Incidence

Incidence of ATT induced hepatitis varies from 3 to 28 % as shown in various studies. WHO reports show that incidence of ATT induced hepatitis is higher in India and China compared to Western countries (2). A meta analysis of 14 published studies from western countries showed a ATT induced hepatitis incidence of 4.38 % (3). One of the largest prospective studies was published by Shang (2011) et al from China(4). In that population based study, 4304 patients who received DOTS treatment were monitored for hepatotoxicity. Only one hundred and six patients developed drug induced hepatitis with an incidence of 2.55%. One possible reason for such a low incidence may be due to DOTS regimen which has been shown in other studies to cause lesser drug induced hepatitis than daily regimen.

On the other hand, studies from India showed an incidence of 16% (a study from North India by Sharma et al) and 10.5% (In another study by Deepak et al). The reason for higher incidence in India and other South East Asian countries compared to other countries is clearly unknown. Possible reasons may be due to ethnic susceptibility, and the presence of more clinical risk factors or genetic polymorphisms.

It is possible that drug induced liver injury may be over diagnosed. In a prospective study published by Davern et al(5), 318 patients with drug induced liver injury was studied. 50 patients (16%) tested positive for anti- HEV IgG and 9 patients had developed anti- HEV IgM. Out of these patients, 4 patients tested positive for HEV genotype 3. Hence serological tests are recommended to rule out acute viral hepatitis if there is high clinical suspicion so that unnecessary interruption of treatment can be prevented.

Hepatic adaptation

About 20 percent of patients may have transient elevation in liver enzymes soon after initiation of anti-tubercular drugs secondary to hepatic adaptation(6). Exposure of the individuals to various drugs can cause physiological adaptive responses. Genes that regulate anti-inflammatory, antioxidant and anti-apoptoptic pathways may be induced which attenuate toxin related changes. This may stimulate protective adapation and hepatocyte proliferation. Hence asymptomatic transient elevation in liver enzymes may be common, especially alanine transaminase (ALT) during the first few weeks after starting treatment which results secondary to non-progressive injury to mitochondria and cell membranes. Hence hepatic adaptation can be misdiagnosed as drug induced liver injury which can further lead to interruption of treatment. Hence differentiating between hepatic adaption and drug induced liver injury is essential. Currently there are no laboratory assays available which can differentiate both. Hence drug induced liver injury should be diagnosed based on clinical features and careful monitoring of liver enzymes.

Mechanisms of drug induced liver injury

The mechanisms of drug induced liver injury(7) can be classified into three types.

- Immunological – associated with fever, eosinophilia, rash and abnormal liver function tests (classically associated with Rifampicin induced hypersensitivity).
- Idiosyncratic
- Dose dependent – for example, isoniazid induced hepatitis.

Risk factors Studies

Many studies to identify clinical risk factors of developing drug induced liver injury have been conducted(8,1,9,10). Risk factors which were found to be significant from

those studies include: demographic factors (such as age, female sex)(9,11–18), abnormal baseline liver function tests(9,12,15,16) , underlying liver disease (such as Chronic Hepatitis B, C infection and significant alcohol intake)(19,14,17,20–22), HIV infection(29–32), malnutrition(9,12,16,25,26) and extent of tuberculosis. Another interesting risk factor which was significant in one of the studies was weight loss during treatment. Warmelink et al published a retrospective study(27) in British Journal of Nutrition (2011) in which they showed weight loss of more than 2 kilograms in the first 4 weeks of treatment was a significant risk factor for developing ATT induced hepatitis (Odds ratio 211 95%CI 36-1232 ,p-value < 0·001). However there is no scoring system available till date for predicting ATT induced hepatitis to enable careful monitoring and early identification.

Indian studies

Indian studies on risk factors for drug induced hepatitis were published as early as 1981. One of those early studies was published by Pande et al which was a case control study from AIIMS, Delhi(28). Eighty six consecutive patients were enrolled and compared against 406 controls. Older age group, slow acetylators, extensive disease, hypoalbuminemia and high alcohol intake were found to be significant risk factors for drug induced hepatitis.

In a prospective study by Sharma et al published in 2002 ,the incidence of drug induced liver injury was 16.5% (16). Risk factors found to be significant were older age, advanced disease and baseline hypoalbuminemia. In another study published by Singla et al, significant risk factors for drug induced liver injury were age more than 35 years, hypoalbuminemia and mid arm circumference less than 20 centimeters(29). Risk factors like advanced age, hypoalbuminaemia, high alcohol intake, slow acetylator phenotype, and extensive disease predisposed the patients to drug induced liver injury according to the study by Pande et al(28).

One of the recent studies from India was published in Journal of Postgraduate Medicine (March 2014) by SM Pore from Maharashtra(30). It was a retrospective study including 893 patients admitted in a tertiary care hospital from 2005 to 2009. Baseline characteristics revealed predominant male population (70%) with mean age of 40 years. Significant number of people had past history of ATT intake (30.36% among cases and 44 % among controls). 56 patients developed drug induced hepatitis with most of them requiring hospitalization. Incidence of drug induced hepatitis from that study was 6.27% which was lower compared to other Indian studies. Significant risk factors for drug induced hepatitis from univariate analysis were female gender, past history of ATT intake and alcohol abuse. However in the multivariate analysis, only female gender and alcohol abuse were significant. Effect of other risk factors on drug induced hepatitis like Hepatitis B and C virus infection, pregnancy, hypoalbuminemia and malnutrition were not studied probably because of inadequate data.

Daily versus intermittent DOTS regimen

Among the various risk factors studied, one of the modifiable risk factors is the treatment related risk factor. Both thrice weekly DOTS and daily regimens are commonly used in Indian population. Whether daily regimen predisposes the patients more to ATT induced liver injury than DOTS regimen is always a concern. There are few studies which compared these two regimens worldwide. In an Indian study (31) published by Mandal et al in 2012, administration of daily regimen predisposed the patients more to drug induced liver injury as compared to intermittent DOTS regimen (7.5% versus 2.32 %). This study included only patients with sputum positive pulmonary tuberculosis and was followed only till completion of intensive phase. Both regimens had equal sputum conversion rate at the end of intensive phase. However default rate is more in the DOTS group (9.3% versus 5%). However we need larger studies and more information about relapse rate before favoring DOTS regimen.

Genetic susceptibility to Drug induced hepatitis (DIH)

The variability in susceptibility to drug induced hepatitis despite the similar doses raises the issue of genetic susceptibility to drug induced hepatitis. Pharmacogenomic variability in drug induced metabolism is well recognized for alcohol, anti retroviral therapy and cancer chemotherapy. Could such a mechanism explain the idiosyncratic nature of ATT induced hepatitis as well as population based differences? Various gene based association studies revealed ATT related liver Injury susceptibility genes such as N-acetyl transferase (NAT2), Cytochrome P450 2E1 (CYP2E1), glutathione S transferase M1 (GSTM1) glutathione S-transferase T1 (GSTT1)and HLA-DQA1/-DQB1(11,32–39). However they also indicate variability between studies and populations.

Mechanism of Isoniazid and NAT2

Anti-tubercular drug induced hepatitis occurs in relation to individual drugs. The mechanism of drug toxicity varies for the different anti-tubercular drugs. This concept is elaborated below in relation to Isoniazid.

Isoniazid (INH) is metabolized by N-acetyl transferase -2 which is involved in phase II biotransformation. INH is mainly inactivated by *N*-acetyltransferase 2 (*NAT2*) mediated acetylation,(40) resulting in acetylisoniazid which is hydrolyzed to acetyl hydrazine and isonicotinic acid. Acetyl hydrazine is either hydrolyzed into hydrazine or acetylated into diacetylhydrazine, a non-hepatotoxic molecule. A small part of INH is directly hydrolysed into hydrazine and isonicotinic acid, and this pathway is greater in slow than in rapid acetylators , oxidized by cytochrome P450 2E1 (CYP2E1) to form hepatotoxic intermediates(41). In slow acetylators, more INH is left for direct hydrolysis into hydrazine(42–44); this also increases the accumulation of acetylhydrazine, which can be converted into hepatotoxic intermediates predisposing to liver injury.

Metabolism of Isoniazid

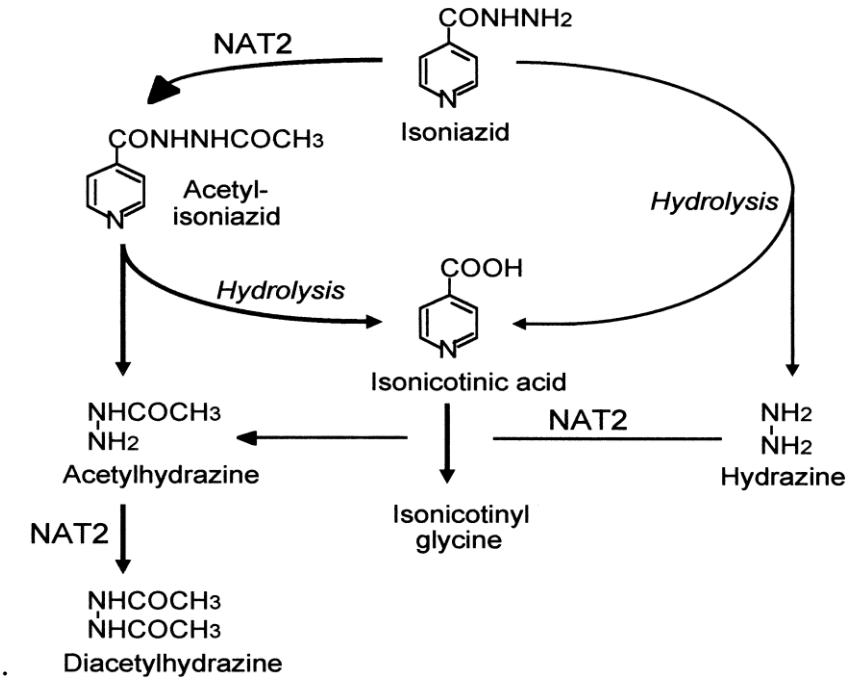

Fig.1 Mechanism of Isoniazid

Therefore slow acetylators may be prone to Isoniazid drug induced hepatitis. Also among the gene based association studies , NAT2 polymorphism has been definitely associated with Isoniazid induced hepatitis (11,34–39,45–48). NAT2 gene is highly polymorphic which is located in the chromosome 8p22. The polymorphism is highly attributed to presence or absence of single nucleotide polymorphisms (SNP). Genetic polymorphisms of INH metabolizing enzyme, NAT2 (N-acetyl transferase 2 (NAT2) have been studied which suggest that slow acetylators are more prone to Drug induced hepatitis than rapid acetylators (11,34–39,45–48).

India is known to exhibit vast diversity in socio-ethnic groups. Distinct genetic divergence is known to occur between different parts of India. We have known from

population based studies about the genetic divergence leading to different disease conditions like lactose intolerance and sickle cell anemia.

Studies that have looked at NAT2 polymorphism show that incidence of slow acetylators among people in South India was 74 % which is higher compared to other Indian studies (according to a South Indian research article by Anitha et al)(49). Another study by Bose et al(35) shows that there was higher prevalence of NAT2 slow acetylator genotypes among patients with ATT induced hepatitis (70%) compared to those who did not develop hepatitis (44.6 %). For these reasons, based on the data and samples that we have from this study, we are further planning to study the NAT2 genetic polymorphism as a risk factor for Isoniazid induced hepatitis as a second phase of this study.

Types of Hepatitis

Drug induced hepatitis is classified based on abnormality of liver function tests and severity of hepatitis.

- The pattern of drug induced hepatitis may be pure enzyme elevation, hepatitis or cholestasis.
- The severity of liver damage may vary from asymptomatic liver enzyme elevation to acute fulminant hepatitis and chronic hepatitis.

Definition of ATT induced hepatitis

ATT induced hepatitis is defined according to American Thoracic society(6) is defined as

- ✓ Normalization of liver enzymes and resolution of signs and symptoms of hepatotoxicity after withdrawal of all anti-TB drugs, and
- ✓ Presence of at least one of the following:
    - A rise to more than 5 times the upper limit of normal (ULN) level of liver enzymes

- Any increase in more than 3 times the upper limit of normal level of liver enzymes above pretreatment levels together with anorexia, nausea, vomiting, and jaundice.

This definition is based on development of hepatitis coincident following initiation of anti-tubercular drugs, normalization of liver function tests after withdrawal of drugs and exclusion of other causes of hepatitis (for example –viral hepatitis). Since multiple anti-tubercular drugs are used simultaneously, it is difficult to identify the incriminating drug except by rechallenge.

Monitoring after initiation of treatment

The British Thoracic Society and Task Force recommend baseline liver function tests before initiation of treatment. The American Thoracic Society do not recommend baseline liver function test before starting treatment. Regular monitoring of liver functions tests is required in patients with known chronic liver disease. American Thoracic society recommends regular monitoring for patients more than 35 years of age.

Management of ATT induced hepatitis

There are two main approaches to management of ATT induced hepatitis(6,50–52):

- Reintroduction of full dose of one drug at a time preferably Rifampicin followed by Isonizaid and Pyrazinamide (according to ATS guidelines).
- Reintroduction of escalating doses of one drug at a time (according to British Thoracic Society guidelines).

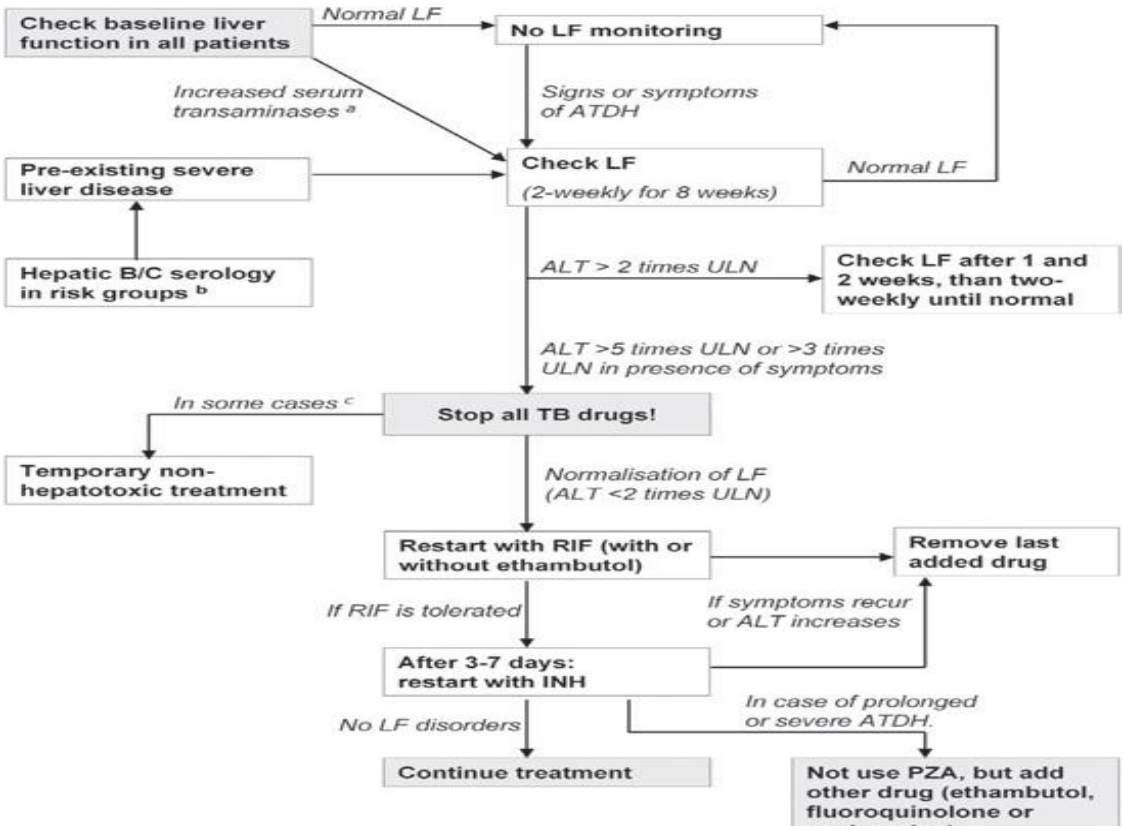

Fig. 2 Management ATT induced hepatotoxicity

There are few studies which compared different methods of reintroduction of anti-tubercular drugs. A study conducted by Sharma et al in a North Indian tertiary hospital compared the safety reintroduction of drugs among 3 groups, (Group I – all three drugs at full doses simultaneously, Group II – according to ATS guidelines , Group III – according to BTS guidelines). The result of this study was that the recurrence of hepatotoxicity was not significantly different between 3 groups(53).

In August 2014, a prospective study was published from Karachi by Zuberi et al comparing the reintroduction guidelines for anti-tubercular driung induced hepatitis by the British Thoracic Society and the American Thoracic Society. A total of 325 patients who developed drug induced liver injury were selected. Hepatotoxic anti-tubercular drugs were stopped and put on alternate non-hepatotoxic regimen. Once the liver function test normalized, they were randomly assigned to reintroduction of ATT according to the American Thoracic Society or

the British Thoracic Society guidelines. Primary outcome was the recurrence of ATT induced hepatotoxicity following reintroduction. There was no significant difference in the primary outcome between the two groups (16(9.8%) versus18 (11.1 %). Ease of administration was also evaluated on this study which showed that the American Thoracic Society guideline was easier to follow. Management of ATT induced liver injury according to various guidelines can be summarized as follows:

Table 1:**Summary of management of ATT induced hepatotoxicity based on various guidelines**

Authority	**Monitoring in the presence of risk factors**	**Stopping drugs if clinical features of hepatitis**	**Cut-off levels if symptomatic (ALT)**	**Mode of reintroduction**
American Thoracic Society	Yes	Yes	5 times	One drug at a time full dose
British Thoracic Society	Yes	Yes	5 times	One drug at a time – escalating doses
European Respiratory Society	Yes	Yes	5 times	
Hong kong tuberculosis Service	Yes	Yes	3 times	

Outcome of ATT induced hepatitis

Reintroduction of anti-tubercular drugs following drug induced hepatitis is often successful. In a prospective study conducted in Mumbai by Deepak et al , reintroduction of drugs was successful in 97.4 % of patients(54). This can be possibly explained by hepatic adaptation or tolerance to those drugs. He also compared the outcome of patients recruited before initiation of treatment *with* those who presented with ATT induced hepatitis after initiation of treatment from another hospital. Among the first group, hepatotoxicity was detected earlier than the other group and there were not many hospitalizations, ICU care and no deaths. And the mortality rate was 16.6 % in the second group. Hence this study emphasizes the importance of monitoring symptoms and liver function tests during treatment for early identification of ATT induced hepatotoxicity and prevention of serious complications (acute fulminant hepatitis and death).

Methodology

Design:

This case-control study was nested in a cohort of Department of General Medicine wards and Out-patients and DOTS clinic patients (Community Health and Development) of Christian Medical College, Vellore. It was carried out from April 2014 to May 2015. The study protocol was approved by Institutional Ethics Review Board, Christian Medical College, Vellore. Informed consent was obtained from the patients recruited in the study.

Case Definition

In this study, anti-tubercular drug induced Hepatitis was diagnosed according to American Thoracic Society definition(6)**:**

1. Normalization of liver enzymes and resolution of signs and symptoms of hepatotoxicity after withdrawal of all anti-TB drugs, and
2. Presence of at least one of the following:
    - A rise to more than 5 times the upper limit of normal (ULN) level of alanine transaminase (ALT) and/or aspartate transaminase (AST)
    - Any increase in more than 3 times the upper limit of normal level of AST and/or ALT above pretreatment levels together with anorexia, nausea,vomiting, and jaundice.

If the LFT returned to normal after stopping hepatotoxic drugs, the hepatitis was attributed to hepatotoxic drugs.

In patients who liver enzymes did not normalize even after stopping ATT, subsequent blood tests was done to rule out Acute viral hepatitis (A,B and E).

Enrollment:

All patients who were newly diagnosed to have tuberculosis and undergoing treatment in the respective units were enrolled in this study after a written consent by the principal investigator. Detailed history and clinical examination were done at baseline for all the recruited patients. All the relevant clinical data including age, gender, height, weight, site of tuberculosis, mode of diagnosis, extent of tuberculosis, alcohol ingestion underlying liver disease, presence of co-existent HIV infection, diabetes mellitus were recorded in the patient enrollment proforma (See Appendix) . Baseline laboratory tests were performed including blood sugars, liver function tests, hepatitis B surface antigen (HBsAg), anti-hepatitis C antibody (HCV antibody) and HIV ELISA. Enrollment of patients from DOTS clinic (CHAD) and follow up were done with the help of co-investigator.

Eligibility criteria

All patients newly diagnosed to have tuberculosis, started on anti-tuberculosis treatment and being followed up in General Medicine Outpatient and Inpatient Department in Christian Medical College, Vellore and patients who were started on DOTS treatment at the Community Health and Development (CHAD) hospital which is part of the Community Health department were eligible for this study. All patients who present with suspected ATT related hepatotoxicity (diagnosed and started on treatment in another hospital) were also enrolled in the study.

Exclusion criteria

- Patients with abnormal baseline LFT (increased enzymes or bilirubin).
- Patients who did not give informed consent.

Cases and Controls

All the patients who developed anti-tubercular drug induced hepatitis were classified as group A. The control group consisted of patients on treatment who did not show any evidence of anti-tubercular drug induced hepatitis, selected from the same cohort. All controls as defined above were classified as group B. All patients who presented with suspected ATT related hepatotoxicity (diagnosed and started on treatment in another hospital) were included in the study as Group C.

The incidence of ATT induced hepatitis was obtained from the cohort. The identification of risk factors of ATT induced hepatitis was based on a case control design nested in the cohort study. A descriptive study of clinical profile and outcome of patients with ATT induced hepatitis was also conducted.

Diagnosis of tuberculosis

Mode of diagnosis varied depending upon the suspected site of tuberculosis:

- **Pulmonary Tuberculosis was classified as follows:**
    - Sputum positive if sputum smear showed Acid fast Bacilli
    - Sputum negative if AFB smears were negative but symptoms and radiological features were suggestive of tuberculosis.
- **Tuberculosis meningitis** was diagnosed as PRESUMPTIVE (based on duration of illness, CSF findings (lymphocytic pleocytosis with increased protein and low sugar and radiological features) or DEFINITE (also positive culture).
- **Pleural and peritoneal tuberculosis** was diagnosed based on fluid analysis and /or caseating granulomas on histology or positive culture.
- **Tuberculosis of the lymph node** was diagnosed based on imaging, caseating granulomas on histology or positive culture

- **Tuberculosis of bone and spine** was diagnosed based on histopathology / culture from the suspected area.
- **Genitourinary tuberculosis** was diagnosed based on positive urine AFB smear and /or culture.
- **Disseminated tuberculosis** was defined as radiological features of military or involvement of 2 or more different sites.

Patients who were empirically started on anti-tubercular treatment were also included in the study.

Follow-up of patients on treatment

All patients who fulfilled the inclusion criteria were enrolled by the principal investigator after the informed consent. Patient enrollment form was filled by the principal investigator.

In this cohort, patients on anti-tuberculosis treatment were followed up regularly as follows.

All patients were clinically assessed for symptoms of hepatitis at every visit until completion of treatment by the treating physician. All patients were educated regarding the clinical features of hepatitis (anorexia , nausea , vomiting and jaundice) and report immediately if they develop so.

- All the treating physicians were informed in person and given an electronic alert through Clinical workstation (Electronic Medical Record System of Christian Medical College) to inform the Principal investigator if patients develop ATT induced hepatitis. When the patient was being seen by the treating doctor, an electronic alert appears on the screen requesting them to contact the Principal Investigator.
- Investigations and OP visits of the patients were electronically tracked using the Clinical workstation (CMC EMR) by the principal investigator every 2 weeks or more

frequently if needed. If patients developed ATT induced hepatitis, the principal investigator was contacted by the treating physician.

- ❖ Once the patient developed ATT induced hepatitis, he/she was regularly followed up by the principal investigator in every OP visit till the completion of treatment.
- ❖ Detailed assessment of patients, review of OP chart notes and filling up proforma was done by the Principal Investigator.
- ❖ All the patients who had lost to follow up were contacted later by the principal investigator through telephone. The reasons for lost of follow up, treatment history and complication related to treatment were obtained.

Severity of hepatitis

The degree of severity of hepatotoxicity was assessed by the peak level of serum transaminases and classified according to the World Health Organization Toxicity Classification Standards(6).

Mild - Elevations of AST and/or ALT to 3–5 times the ULN (121– 200 UI/l)

Moderate - 5–10 times the ULN (201–400 UI/l)

Severe - 10 times the ULN (>400 UI/l).

Type of hepatitis

It was sub-classified as follows:

- ✓ **Anicteric hepatitis**

  Elevation of liver enzymes without increase in bilirubin levels

- ✓ **Icteric hepatitis**

  Elevation of liver enzymes with increase in bilirubin levels.

- ✓ **Drug induced cholestasis**

  Direct hyperbilirubinemia without increase in liver enzymes.

- ✓ **Drug induced hepatitis with hepatic decompensation**

  Prolonged PT, low albumin, ascities, encephalopathy

Risk factors (predictors) of DILI

- ➢ **The clinical risk factors were assessed as follows :**

  Age, sex, BMI, underlying liver disease , HIV infection, significant alcohol intake, site and severity of illness, abnormal LFT during initiation of treatment, pregnancy, MDR tuberculosis, past history of anti tubercular treatment, DOTS regimen versus daily regimen.

- ➢ **Genetic :**

  NAT2 polymorphism (to be studied subsequently)**.**

All patients who were diagnosed to have Anti-tuberculosis drug induced hepatitis had the proforma section on drug induced hepatitis filled up. The diagnosis of drug induced liver injury was made as defined above. All hepatotoxic drugs were stopped and a non-hepatoxic regimen was continued.

Reintroduction of drugs after Drug induced liver injury

Once the liver function tests normalized (at least enzymes less than 2 times the upper limit of normal), patients were re- introduced with first line regimen. The treating physician decided regarding mode of re-introduction, either full dose of one drug at a time or an escalating dose introduction or all the three drugs together.

Once the drug was re-introduced, the patients were closely monitored for clinical features of hepatitis and regular monitoring of LFT was done. If symptoms recurred or LFT abnormalities increased, the last drug added was stopped.

At Christian Medical College, Pyrazinamide(PZA) was usually started after Rifampicin(RIF) and Isoniazid(INH). If RIF and INH were tolerated, and hepatitis was severe, it was assumed that PZA was the responsible drug and rechallenge with this drug was generally avoided. However the choice of order of reintroduction was decided by the treatment physician.

Identifying the likely drug that induced drug induced hepatitis

If reintroduction of a particular drug resulted in increase in liver enzymes, then that drug was likely to be the cause of drug induced hepatitis.

In case if the patients did not tolerate all three drugs during reintroduction, the drug which caused hepatotoxicity initially was unknown (Group X)

In case re-challenge of drugs did not take place (for example underlying chronic liver disease or failure of normalization of LFT), then again the incriminating drug cannot be identified (Group X)

Outcome of patients with ATT induced hepatitis

The outcomes measures were defined in terms of Tuberculosis and Hepatitis.

The hepatitis outcome were measured as follows :

- Severity of hepatitis and type of hepatitis (see definitions)
- Time period for normalization of LFT
- Requirement of hospitalizations and ICU care
- Case fatality
- Number of first line drugs successfully reintroduced

The outcome of tuberculosis were measured as

- whether the patient had completed the treatment course
- whether the patient was cured of tuberculosis (For example, improvement of clinical features in TB meningitis or sputum AFB negativity and improvement in Chest X ray in patients with Sputum Positive Tuberculosis or resolution of TB pleural effusion as seen in Chest X ray)
- Duration of treatment
- rechallenge regimen used.

Sample size calculation

Sample size was calculated as follows:

- For the incidence of ATT induced hepatitis, the sample size was calculated from the following study:

  Sharma SK, Balamurugan A, Saha PK, Pandey RM, Mehra NK. Evaluation of clinical and immunogenetic risk factors for the development of hepatotoxicity during antituberculosis treatment. Am. J. Respir. Crit. Care Med. 2002 Oct 1;166(7):916–9.

  Sample Size = $4pq/d^2$

  p = 16% d=3

  Sample Size calculated = 600

- **Clinical Risk factors**

  The sample size was calculated as per the formula for the case – control study. The power of the study was taken as 80% with alpha error of 5 %. The following study was chosen for calculation of sample size.

One of the significant risk factor for ATT induced hepatitis shown in the previous study was baseline hypoalbuminemia (albumin<3.5g/dl) before initiation of treatment. The sample size was calculated by the following formula:

$$\textbf{Sample size} = (Z\alpha + Z\beta)^2\, 2p * q / (p1-p2)^2$$

The sample size calculated was 220.

Statistical methods

Incidence of ATT induced hepatitis was calculated from the cohorts with 95% confidence interval. The risk factors for ATT induced hepatitis will be identified by bivariate analysis and logistic regression analysis with odds ratio and 95 % confidence interval. A *P*-value less than 0.05 are considered significant. A *Z*-value greater than 1.96 is considered significant at 95% confidence interval.If the event rate is less than 10%, then LR analysis with log link will be done.

Results

A total of three hundred and ninety three patients who were initiated on anti-TB treatment were eligible for our study. They were recruited in the Department of General Medicine wards and Out-patients and DOTS clinic patients (Community Health and Development hospital) of Christian Medical College, Vellore. This study was carried out from April 2014 to May 2015. 14 patients were excluded from the study since they did not give consent. Out of 393 patients, 5 patients presented to the hospital with drug induced liver injury and classified as Group C. Remaining 388 patients belonged to the cohort of patients with newly diagnosed with tuberculosis who were initiated on anti-TB treatment.

Strobe Figure

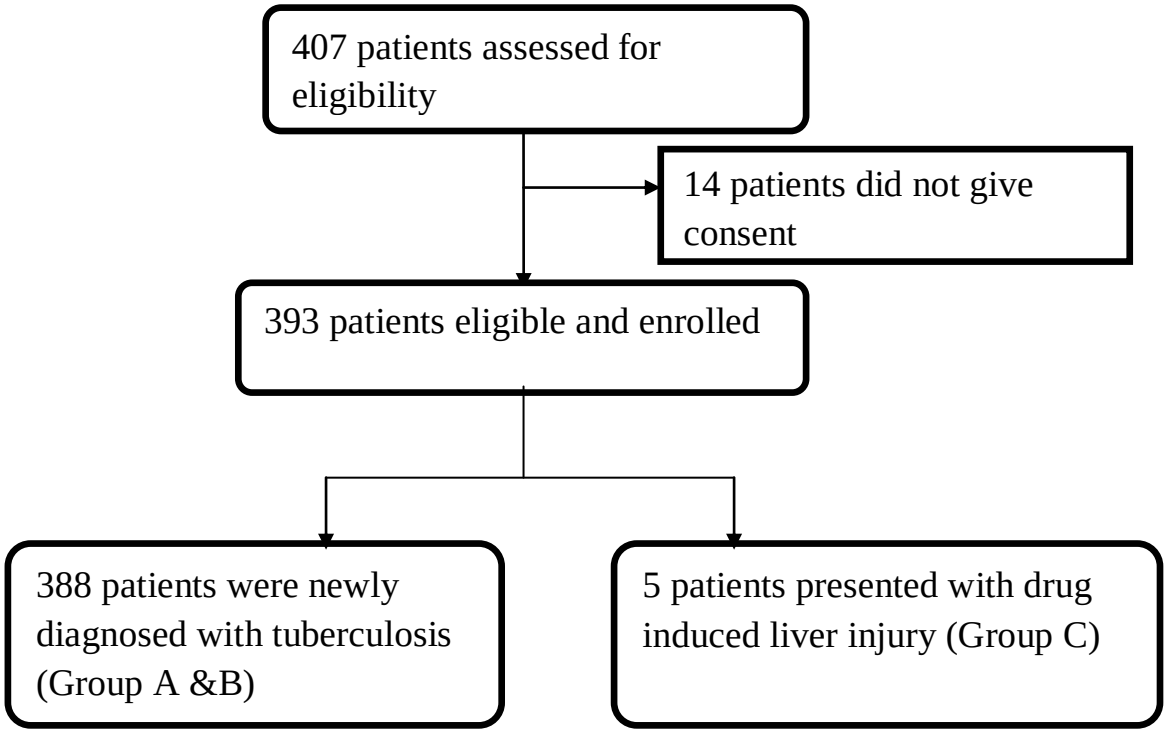

Fig.3 STROBE Figure

Baseline characteristics of the patients

Out of 393 patients, 308 patients were enrolled from the Department of General Medicine, Christian Medical College Hospital (CMC), Vellore and 85 patients from the DOTS clinic at Community Health and Department hospital, CMC Vellore.

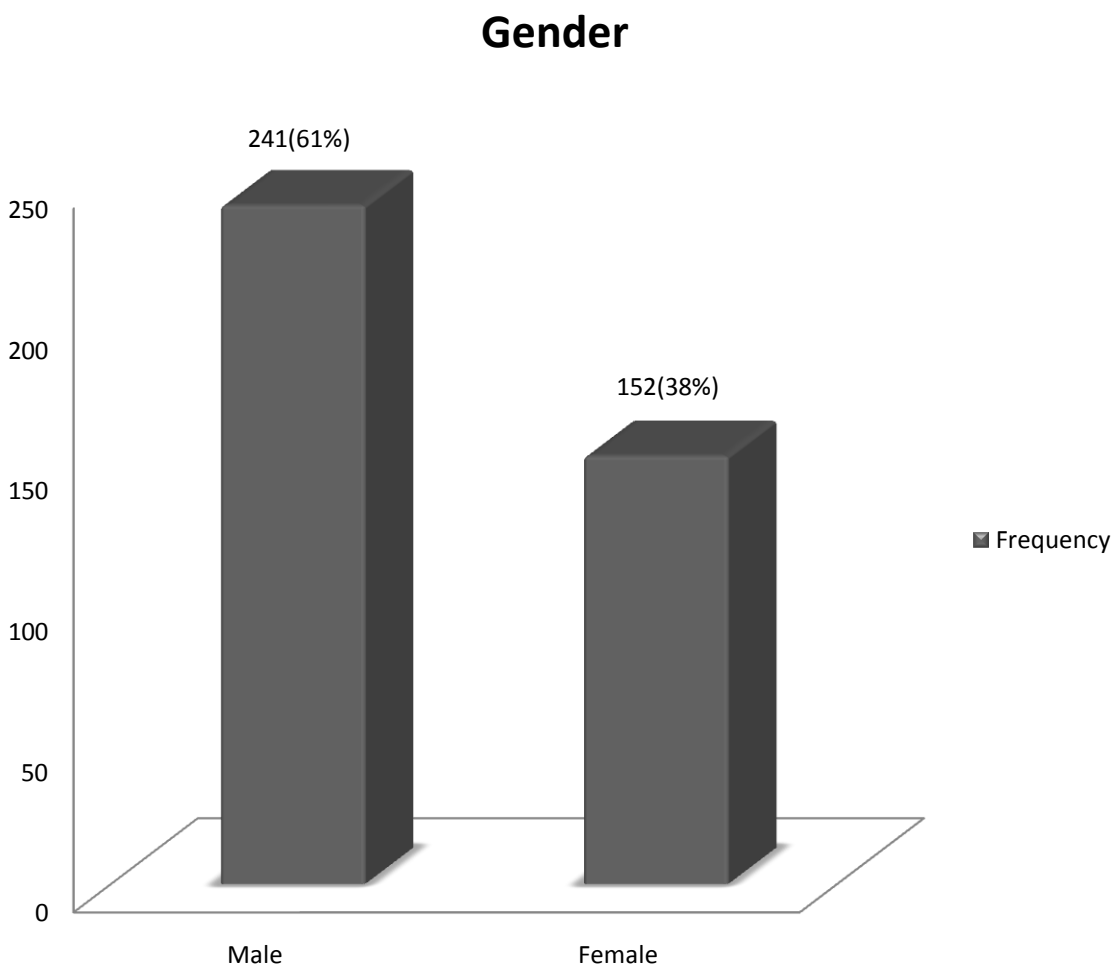

Fig.4 Gender distribution of the patient cohort

In the cohort of 393 patients, 241 patients (61%) were male and 152 patients (38%) were female (Fig.4).

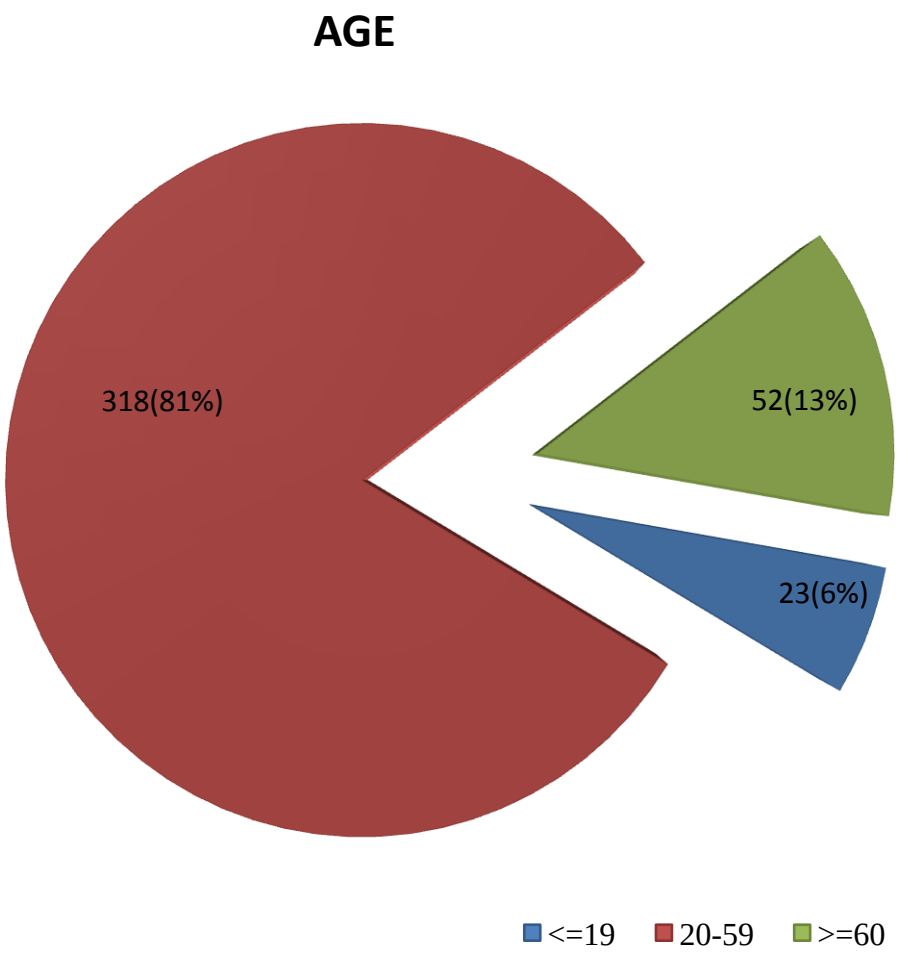

Fig. 5 Age distribution of the patient cohort

Most of the patients (81%) were in the age group 20-59. Twenty three patients (6%) belong to younger group while 52 patients (13%) belonged to older age group (Fig.5). Data on Body mass index was available for 362 patients . One hundred and seventy six patients (45%) had normal body mass index, and 134 patients (34 %) of enrolled patients had a low body mass index (<18.5) and 50 patients (15%) were overweight at enrollment (Fig. 6).

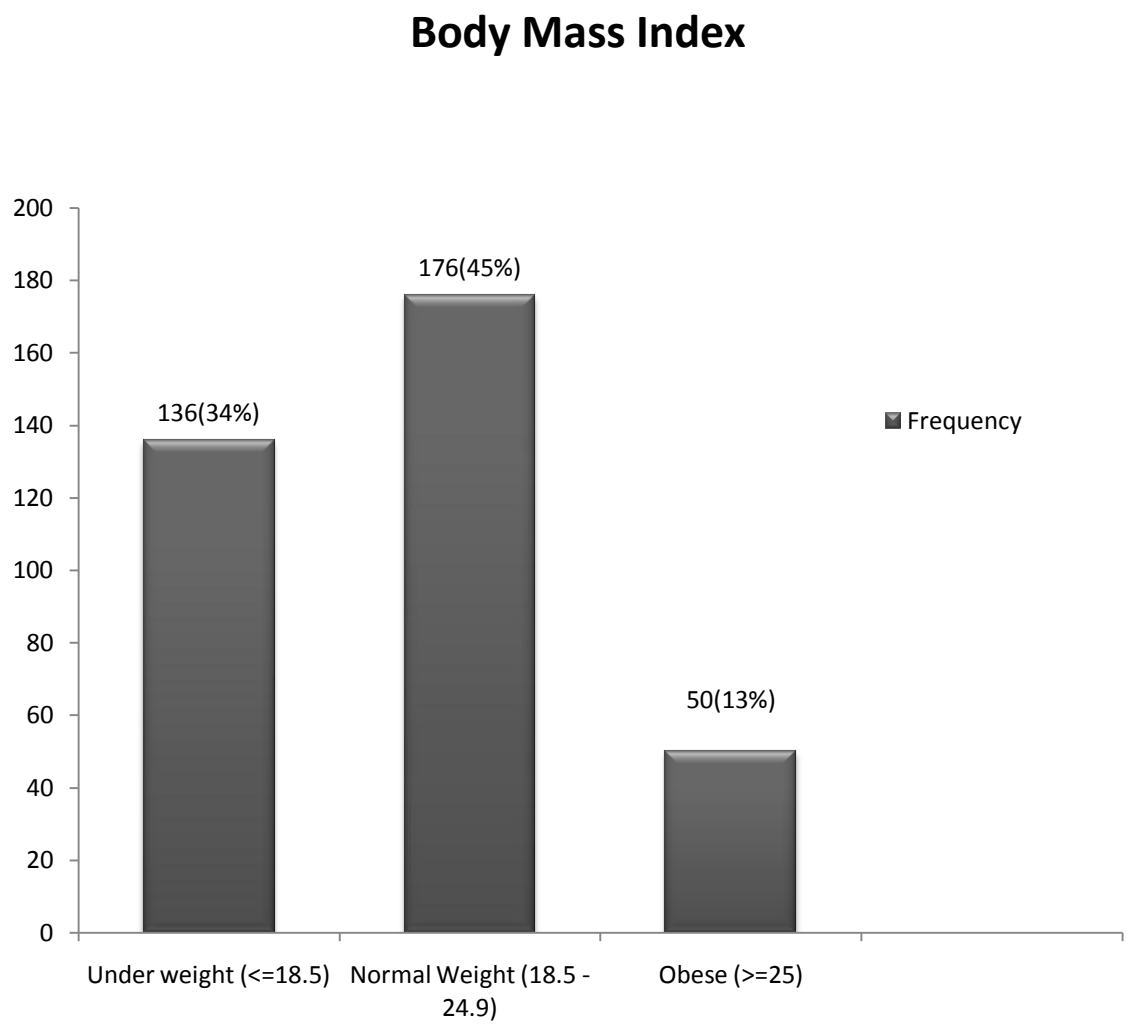

Fig.6 Body mass index (BMI) of the patient cohort.

HIV infection was found to be positive in 42 patients. Diabetes mellitus was present in 97 patients (24.7%).Among diabetic patients, mean HbA1c was 8.7 % suggesting uncontrolled diabetes at the time of diagnosis. HbA1c measurements ranged from 5.1 to as high as 14.6 %. Risk factors for developing chronic liver disease were also studied in this cohort such as hepatitis B and C and history of alcohol intake. Detailed history of significant alcohol intake could not be obtained from all the patients. A hundred patients (25.4%) had at least one risk factor for developing chronic liver disease. History of alcohol intake (both current and past) was documented in 93 patients (23.7%). However clinical features of chronic liver disease

were present only in 14 patients (3.6%). Twenty three patients (5.9%) had past history of jaundice (Table 2). Among 390 patients, (9.4%) of them had past history of intake of anti-tubercular drugs. One hundred and fourteen patients (29%) were started on DOTS regimen with majority of patients enrolled from Community Health and Development (CHAD) DOTS clinic. The remaining patients (71%) were treated with weight based daily regimen from the Medicine outpatient department.

Table 2.Baseline characteristics of patients

	Number of patients	**Percentage (%)**
Diabetes mellitus	97	24.7
HbA1c	Mean-8.75 % (Range 5.1-14.6)	
Hypoalbuminemia	177	45.0
HIV infection	72	18.3
Risk factors for liver disease	100	25.4
Alcohol intake	93	23.7
Chronic liver disease	14	3.6
Past history of jaundice	23	5.9
Pregnancy	1	.3
Past history of ATT intake	37	9.4
DOTS regimen	114	29
Daily regimen	279	71

Comparison of baseline characteristics of patients on daily and DOTS regimen

While comparing the patients on daily and DOTS regimen, mean age and gender distribution were similar. More patients on DOTS regimen (97.4%) had localized disease when compared with patients on daily regimen (33%). HIV infection was more commonly seen in patients on daily regimen (22.4% Vs 8.8%). Fifty one percent of patients on DOTS regimen were underweight when compared to daily regimen (33%). Other baseline characteristics were similar in both groups. To summarize, patients on DOTS regimen had lower rates of HIV infection and disseminated disease but had greater undernutrition when compared with patients on daily regimen.

Table 3: Baseline characteristics of patients on daily and DOTS regimen

	Daily (%)	**DOTS (%)**
Age(mean)	41.7	40.4
Sex **Male** **Female**	 167(60) 112(40)	 74(65) 40(35)
BMI **Under weight** **Normal weight** **Obese**	 **90(33)** 141(52) 41(15)	 **46(51**) 35(39) 9(10)
Extent **Local** **Disseminated**	 **170(61)** 109(39)	 **111(97.4**) 3(2.6)
Diabetes Mellitus	65(23%)	32(28%)
HIV infection	**62(22.4%)**	**10(8.8%)**
Alcohol intake	50(18%)	33(29%)
Hepatitis B	5(1.8%)	3(2.6%)
Chronic Liver Disease	13(4.7%)	1(1%)
Past history of jaundice	18(6.5%)	5(4.4%)
Hypoalbuminemia	133(49%)	44(39%)
Past history of ATT intake	35(12.6%)	2(6.5)

Diagnosis and Extent

Majority of the patients (38.9 %) patients had sputum positive pulmonary tuberculosis. 101 patients (25.7%) had disseminated tuberculosis and 54 (14%) patients had tuberculous meningitis. Different sites of tuberculosis are summarized below in the table 4.

T**able 4 Distribution of tuberculosis according to anatomical site**

Site of TB	**Number of patients**	**Percentage (%)**
Sputum positive pulmonary	153	38.93
Disseminated	101	25.70
Meningitis	54	13.74
Lymphadenitis	35	8.91
Pleural effusion	13	3.31
Sputum negative pulmonary	9	2.29
Peritonitis	7	1.78
Spine	6	1.53
Osteomyelitis	5	1.27
Central nervous system	3	0.76
Synovitis	3	0.76
Others	2	0.51
Miliary	1	0.25
Genito urinary	1	0.25

Among 393 patients in our cohort, 281 patients (72%) had localized disease and 112 patients (28%) had disseminated disease (fig.7).

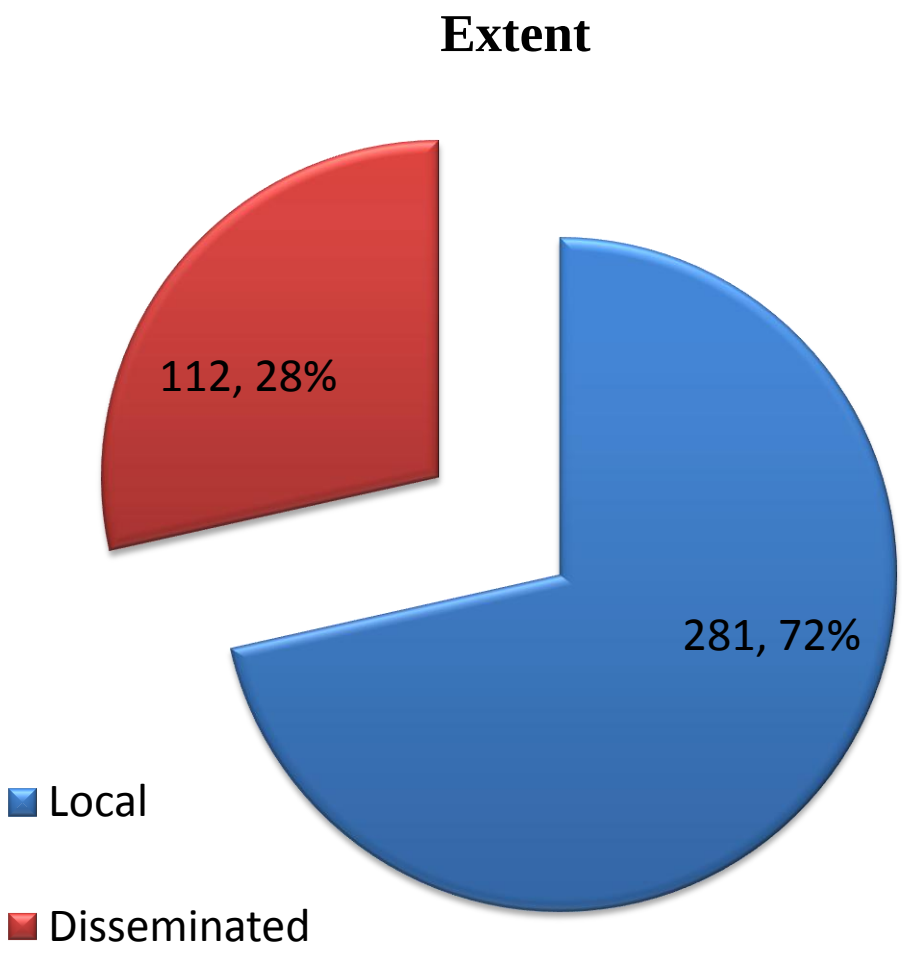

Fig. 7 Extent of tuberculosis in the patient cohort

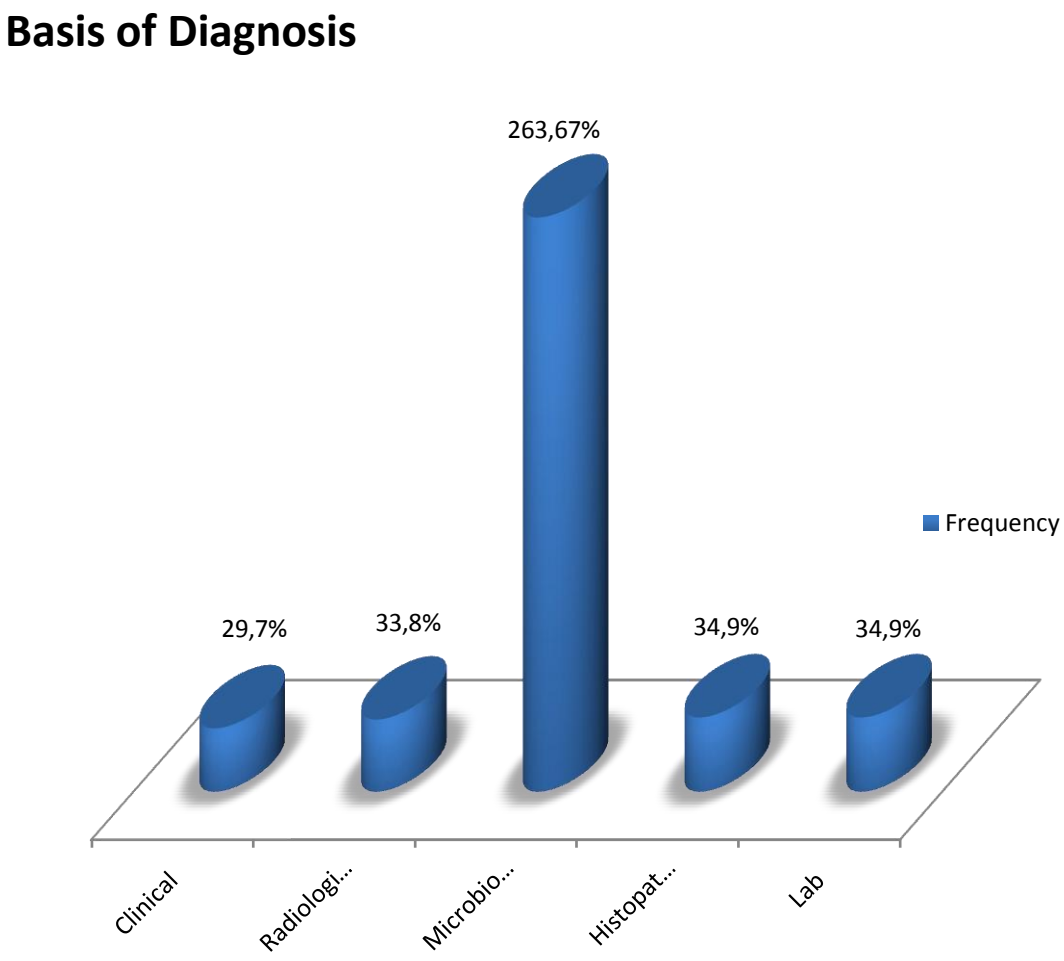

Fig.8 Method of confirmation of TB diagnosis in patient cohort (393 patients)

Diagnosis was made based on microbiological basis in most of the patients (66.9%). This was followed by histopathology, lab, radiological and clinical diagnosis respectively (Fig.8).

Risk factors for liver disease

Two hundred and ninety three patients (74.6 %) did not have any known risk factors for chronic liver disease of alcohol use, Hepatitis B or C infection. History of alcohol intake was present in 83 patients (21.1%). However quantification of alcohol intake could not be obtained in all the patients. Hepatitis B and C infection were present in 8 and 2 patients respectively.

Table 4.Risk factors for chronic liver disease in patient cohort

Risk factors	**Number of patients**	**Percentage %**
None	293	74.6
Alcohol intake	83	21.1
Hepatitis B	8	2
Hepatitis C	2	0.5
Others	4	1

TB PCR and Microbiological culture

Gene Xpert TB PCR was done in 234 patients out of whom 142 patients (47 %) had positive results. Rifampicin resistance was not detected in 116 patients (84 %) where as in 16 patients (12%), rifampicin resistance was detected (Fig.9).

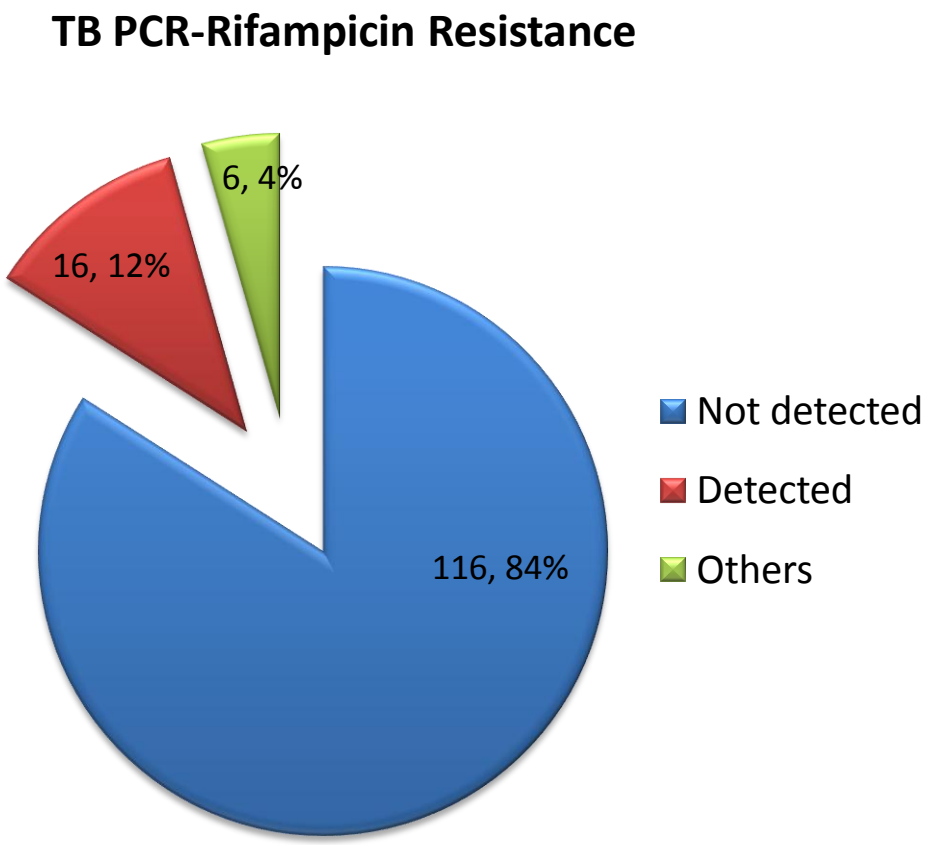

Fig.9 Rifampicin resistance according to Gene Xpert TB PCR in patient cohort

Mycobacterial culture was done in 275 patients out of whom 120 patients (39 %) had positive culture (fig.8). Of 95 patient with drug succeptibility testing results, 67 patients (70.5% had Pan-susceptible tuberculosis and 28 (29.5%) had drug resistant Mycobacterium tuberculosis. Eleven patients had MDR tuberculosis . Four patients had XDR tuberculosis. Izoniazid monoresistance was seen in 8 patients (2.6%) and rifampicin monoresistance in 2 patients.

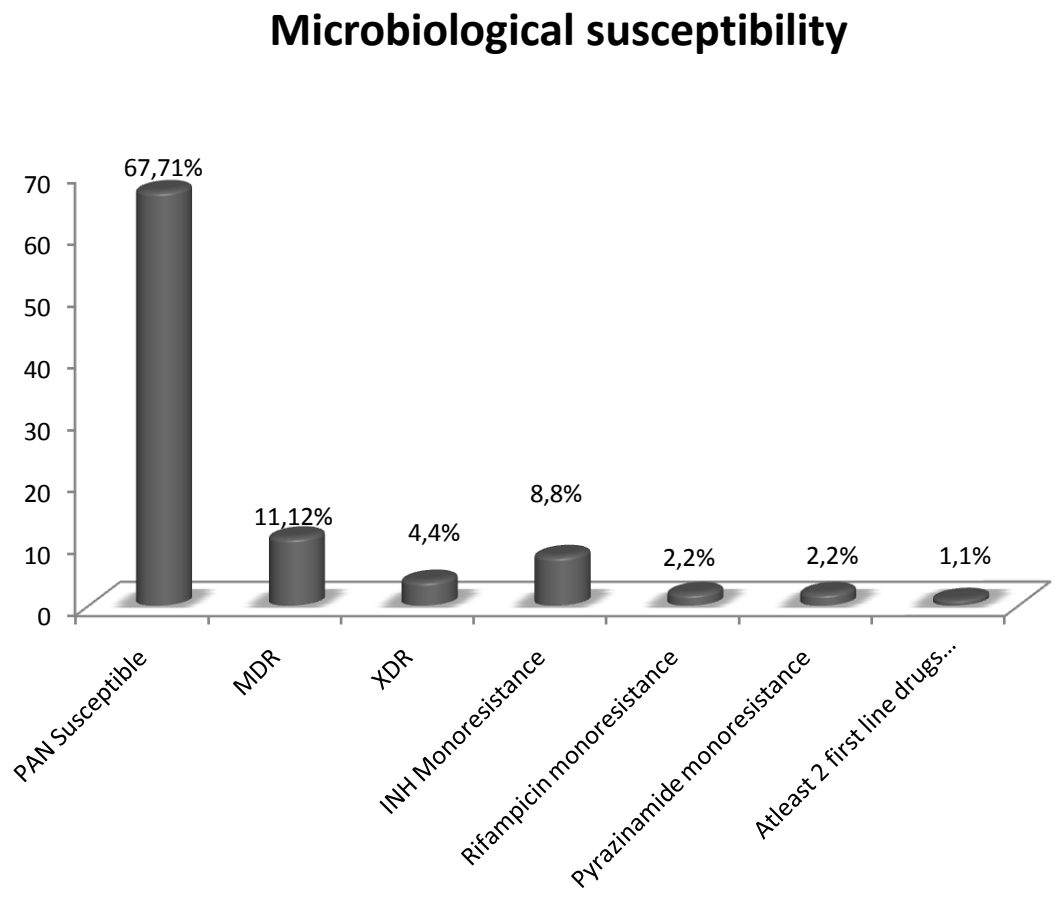

Fig. 10.Microbiological ssceptibility results among patient cohort

Current status and follow up

All patients were followed up till 1st week of August 2014. A hundred and eight patients (29%) had successfully completed treatment and cured. A hundred more patients are still under treatment. One hundred and twenty eight patients (34.4%) had lost follow up in our hospital (fig.11). Mortality rate was 7.5% (28 patients). Six patients were referred to local DOTS centre for continuation of treatment.

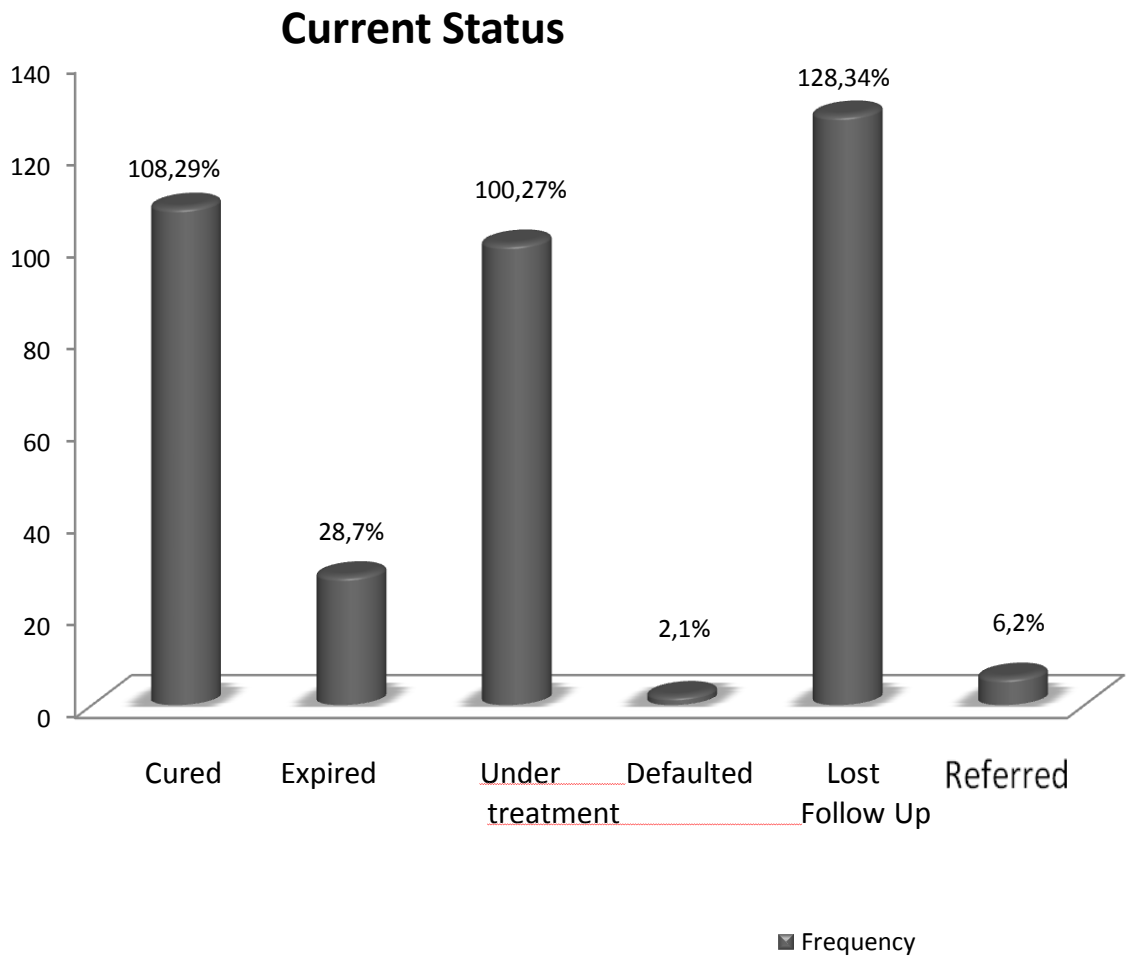

Fig.12 Current status of TB outcomes in patient cohort

Incidence of drug induced liver injury

During follow up, 43 patients developed anti-tubercular drug induced liver injury during the course of treatment. The incidence of drug induced liver injury was calculated after excluding the patients from Group C .

Hence after excluding these five patients, 38 patients out of 388 patients from the cohort developed drug induced liver injury while on treatment. Therefore incidence of anti-tubercular drug induced liver injury was calculated as 9.7 %.

\

Incidence of ATT induced hepatitis = 38/388 = 9.7% (95% C.I 7-13.2%)

Incidence in DOTS regimen 3.5% (95% C.I 2.4%-4.8%)

Incidence in daily regimen 14% (95%C.I 7.9 – 22.4%)

Predictors of Drug induced liver injury (DILI)

Predictors of drug induced liver injury (DILI) were classified into three categories

1) Patient related background factors
2) Disease related
3) Treatment related

Background factors

- ✓ Gender
- ✓ Age
- ✓ BMI
- ✓ Hypoalbuminemia
- ✓ Liver disease
- ✓ Alcohol intake
- ✓ Past history of jaundice
- ✓ Past history of ATT intake
- ✓ Pregnancy
- ✓ Genetics like NAT2 polymorphisms

Disease related

- ✓ Local or disseminated disease
- ✓ HIV co-infection

Treatment related

- ✓ DOTS versus daily regimen

Gender

There was no significant difference in gender between cases and controls (p value 0.14). In few published studies, female gender was found to be significant risk factor for drug induced hepatitis(28). However in our study, gender was not a significant risk factor.

Table 5: Gender distribution and DILI

Gender	DILI (%)	No DILI(%)	Odds Ratio	95% C.I.
Male	22(51)	219(63)	0.627	0.332 -1.184
Female	21(49)	131(37)	p-value 0.147	

Age

Table 6: Age distribution and development of DILI

Age	DILI (%)	No DILI (%)
< 19	4 (9)	19(5)
20-59	34(79)	284(81)
> 60	5(12)	47(14) p-value 0.578

Age of the cohort was divided into 3 groups – younger, middle age and older age group. However age was not a statistically significant risk factor on bivariate analysis to cause drug induced hepatitis (p value – 0.578).

Older people are more prone to drug induced liver injury according to previous studies. Hence further analysis was done comparing older age group with all patients <60 years. Still there was no significant association between age and DILI (p value 0.742).

Table 7: Older age group and development of DILI

Age	**DIlLI(%)**	**No DILI (%)**	**Odds Ratio**	**95% C.I**
< 60	38(88)	303(87)	1.18	0.44-3.15
>=60	5(12)	47(13)	p-value 0.742	

Body mass index

Literature review revealed that patients who are underweight are more predisposed to ATT induced hepatitis**.** In our study group, there was no statistically significant association between BMI and DILI (p value – 0.257).

Table 8: Body mass index and development of DILI

Body mass index	**DILI (%)**	**No DILI (%)**
Under weight (<=18.5)	15 (36)	121 (38)
Normal Weight (18.5 - 24.9)	18 (43)	158 (49)
Obese (>=25)	9 (21)	41 (13) p-value 0.257

Further analysis comparing patients who were underweight (<18.5) and all other patients did not show any significant association between weight and DILI (p value 0.792).

Table 9 Underweight and developmental of DILI

Body mass index	**DILI(%)**	**No DILI (%)**	**Odds Ratio**	**95 %C.I**
Under weight (<=18.5)	15 (36)	121 (38)	0.91	0.47-1.79
Others (>=18.5)	27 (64)	199(62)	p-value 0.792	

Similarly obesity was not found to be a significant variable in the development of DILI (p value 0.655).

Table 10 Obese patients and development of DILI

Body mass index	**DILI (%)**	**No DILI (%)**	**Odds Ratio**	**95 % C.I**
Others(<=24.9)	33(79)	279 (87)	0.81	0.32-2.05
Obese (>=25)	9 (21)	41(13)	p-value 0.655	

Past history of ATT intake

Table 11 Past history of ATT intake and development of DILI

Past ATT intake	**DILI (%)**	**No DILI (%)**	**Odds Ratio**	**95 %C.I**
Yes	8(19)	29(11)	1.86	0.79-4.39
No	35 (81)	236 (89)	p-value 0.152	

In patients with drug induced liver injury, 8 (19%) had past history of ATT intake whereas 29 (11%) patients had past history of ATT intake in control group. Prior ATT was not a statistically significant risk factor for development of DILI (p value 0.15).

Past history of jaundice

Twelve percent of cases of DILI had past history of jaundice when compared to 5% of the patients who did not develop DILI. Though this variable was not significant (p value - 0.087), odds ratio was 2.43 suggesting it was close to being significant.

Table 12 Past history of jaundice and development of DILI

Past history of jaundice	DILI(%)	No DILI (%)	Odds Ratio	95 %C.I
Yes	5 (12)	18 (5)	2.43	0.85-6.91
No	38 (88)	332 (95)	p value0.087	

Hypoalbuminemia

Hypoalbuminemia was significantly associated with the development of DILI. Sixty percent of DILI cases had hypoalbuminemia when compared to 44% of the controls who did not develop DILI (p value -0.045, OR1.92, 95%CI 1.01 – 3.68).

Table 13 Hypoalbuminemia and DILI

Hypoalbuminemia	DILI (%)	No DILI (%)	Odds Ratio	95 %C.I
Yes	26 (60)	151 (44)	1.92	1.01-3.68
No	17 (40)	190 (56)	p-value 0.045	

HIV infection

Thirty six percent of DILI cases had HIV infection when compared to 16 % of the controls (without DILI) who had HIV infection. Hence HIV infection was found to be a

significant risk factor for development of DILI. (p value 0.002 ,OR 2.84 , 95 % C.I 1.42-5.67).

Table 14 HIV infection and development of DILI

HIV	**DILI(%)**	**No DILI (%)**	**Odds Ratio**	**C.I**
Yes	15 (36)	57 (16)	2.84	1.42-5.67
No	27 (64)	291 (84)	p-value 0.002	

Site of tuberculosis

Diagnosis was grouped into two categories:

- ✓ Disseminated and severe extra pulmonary disease
- ✓ Pulmonary and non severe extra pulmonary disease.

The incidence of DILI is pulmonary and non severe extrapulmonary TB was – compared to – in patients with disseminated and severe extrapulmonary TB. Disseminated and severe extrapulmonary TB group was a significant risk factor for drug induced hepatitis (70% Vs 44%, p value 0.001, OR 2.971,95 % CI 1.49-5.89).

Table 15 Diagnosis and Development of DILI

Diagnosis	**DILI (%)**	**No DILI (%)**	**Odds Ratio**	**95% C.I**
Disseminated / Severe EP	30 (70)	153(44)	2..971	1.49-5.89
Pulmonary / Non severe EP	13 (30)	197 (56)	p-value 0.001	

Regimen

The only modifiable variable among various risk factors is the treatment related factor. DOTS and daily regimen was compared among cases and controls.

Table 16 Regimen and development of DILI

Regimen	**DILI (%)**	**No DILI (%)**	**Odds Ratio**	**95%C.I.**
Daily	39(14)	240(86)	4.469	1.558-12.814
DOTS	4(3.5)	110(96.5)	p-value 0.003	

Fourteen percent of patients on daily regimen developed jaundice as compared to only 3.5% of patients on DOTS regimen (p value 0.003, OR 4.469, 95% CI 1.56-12.81). Daily TB treatment regimen is significantly associated with development of DILI with an odds ratio of 4.469.

Risk factors for liver disease

Table 17 Risk factors for liver disease and development of DILI

Risk factor for Liver Disease	**DILI** (%)	**No DILI** (%)	**Odds Ratio**	**C.I**
Yes	12 (28)	88 (25)	1.15	0.57-2.34
No	31 (72)	262 (75)	p-value 0.695	

Risk factors for liver disease assessed in our study are alcohol intake, hepatitis B and hepatitis C infection. Twenty eight percent of the DILI group had risk factors for liver disease when

compared to 25% of the patients who did not develop DILI. This difference was not statistically significant (p value 0.695%).

Chronic Liver disease (CLD)

14 patients had underlying chronic liver disease. The incidence of DILI in patients who had chronic liver disease was 12 % when compared to 2.7% of patients who did not have chronic liver disease. Hence presence of chronic liver disease was significant risk factor for DILI (with p value of 0.004 and OR of 4.72).

Table 18 Chronic liver disease and development of DILI

CLD	DILI (%)	No DILI (%)	Odds Ratio	95 %C.I
Yes	5 (12)	9 (2.7)	4.72	1.50-14.82
No	38(88)	323 (97.3)	p-value 0.004	

Extent of tuberculosis disease

Disseminated disease was significantly associated with the development of DILI (46.5 % versus 26 %, p value – 0.006, OR 1.769, 95% CI 1.23- 2.55).

Table 19 Extent and development of DILI

Extent	DILI (%)	No DILI (%)	Odds Ratio	95%C.I
Local	23 (53.5)	258 (74)	1.769	1.23-2.55
Disseminated	20 (46.5)	92 (26)	p-value 0.006	

Pregnancy

Only one patient was pregnant among the cohort. Hence risk factor analysis was not appropriate for this patient.

Table 20 Pregnancy and development of DILI

Pregnancy	Cases (%)	Controls (%)	Odds Ratio	95 %C.I
Yes	0(0)	1(.3)	0.0	0.0
No	43(100)	349(99.7)	p-value 0.726	

History of alcohol intake

Table 21 History of alcohol intake and development of DILI

History of alcohol intake	DILI (%)	No DILI (%)	Odds Ratio	95%C.I
Yes	9 (21)	84 (24)	1.19	0.55-2.59
No	34 (79)	266 (76)	p-value 0.655	

History of alcohol intake (both current and past) was not a statistically significant predictor of liver injury. However detailed quantification of alcohol intake could not be obtained from the patients.

Summarizing, clinical significant predictors of ATT induced hepatitis according to bivariate analysis were HIV infection (OR 2.84), hypoalbuminemia (OR 1.92), underlying chronic liver disease (OR 4.72), daily regimen (OR 4.47) and extent of tuberculosis (OR 1.8).

Multivariate logistic regression analysis for risk factors for development of drug induced hepatitis

Multivariate logistic regression analysis was done according to three models which were discussed earlier. The models analyzed are as follows:

- Model1 – Patient related background variable
- Model 2 –Disease and treatment related variable
- Model 3 – All factors together

The above mentioned models form the conceptual framework(55). This concept was originally drafted by WHO in 2005. This framework for analysis has a conceptual orientation based on potential mechanisms of hepatotoxicity (background variables, TB disease variables and TB treatment variables) . This framework shows how major determinants relate to each other and helps us in better understanding of different determinants. Similar framework has been used in an article published by Patel et al in the Journal of Epidemiology(56).

Table 22 Model 1 including background variables

Risk factor Variables	Unadjusted Analysis (N =393)					Adjusted Analysis		
	Case				p value	OR	95 % CI	p value
	No DILI		DILI					
	N	%	N	%				
Gender :								
Male	219	63	22	51				
Female	131	37	21	49	0.14	1.77	0.85-3.7	0.12
Age:								
<=19	19	5	4	9				
20-59	284	81	34	79	0.57	0.627	0.27-1.4	0.27
>60	47	14	5	12				
CLD :Yes	9	3	5	12				
No	323	97	38	88	0.004	3.78	1.12-12.77	0.032
Hypoalbuminemia								
Yes	151	44	26	60	0.045	2.04	1.01-4.1	0.046
No	190	56	17	40				
BMI								
Under weight	121	38	15	36				
Normal Weight	158	49	18	43	0.257	1.42	0.87-2.32	0.159
Obese	41	13	9	21				
Alcohol Intake								
Yes	266	76	34	79	0.65	1.19	0.47-2.9	0.70
No	84	24	9	21				

In the model 1 which included background variables, significant predictors of drug induced liver injury were hypoalbuminemia and chronic liver disease with OR of 2 and 3.8 respectively.

Table 23 Model 2 including disease and treatment variables

Risk factor Variables	Unadjusted Analysis (N = 393)					Adjusted Analysis		
	Case				p value			
	No DILI		DILI			OR	95 % CI	p value
	n	%	n	%				
HIV								
Yes	57	16	15	36	0.002	2.14	1.03-4.45	0.04
No	236	89	35	81				
Regimen								
Daily	240	69	39	91	0.003	3.23	107-9.7	0.037
DOTS	110	31	4	9				
Extent								
Local	258	74	23	53				
Disseminated	92	26	20	47	0.006	1.5	0.75-3.1	0.23

In the model 2 which examined disease and treatment variables, daily regimen and HIV co-infection were found to be significant predictors. Disseminated disease which was very significant (0.006) during bivariate analysis was not significant during multivariate analysis..

Table 24 - Model 3 All risk factors for DILI together

Risk factor Variables	Unadjusted Analysis (N =393)					Adjusted Analysis		
	Case				p value	OR	95 % CI	p value
	No DILI		DILI					
	n	%	N	%				
Gender :								
Male	219	63	22	51				
Female	131	37	21	49	0.14	1.94	0.89-4.2	0.09
Age:								
<=19	19	5	4	9				
20-59	284	81	34	79	0.57	0.71	0.29-1.7	0.46
>=60	47	14	5	12				
CLD								
Yes	9	3	5	12	0.004	3.50	1.01-	0.04
No	323	97	38	88			12.05	
Hypoalbuminemia								
Yes	151	44	26	60	0.045	1.5	0.73-3.1	0.25
No	190	56	17	40				
HIV :Yes	57	16	15	36				
No	236	89	35	81	0.002	2.19	0.99-4.7	0.05
BMI								
Under Weight	121	38	15	36				
Normal Weight	158	49	18	43	0.257	1.25	0.75-2.09	0.38
Obese	41	13	9	21				

Alcohol intake								
Yes	266	76	34	79	0.65	1.09	0.42-2.8	0.84
No	84	24	9	21				
Regimen								
Daily	240	69	39	91	0.003	1.96	063-6.04	0.24
DOTS	110	31	4	9				
Extent								
Local	258	74	23	53				
Disseminated	92	26	20	47	0.006	1.35	0.64-2.8	0.42

In the model 3 which included all background, TB disease and treatment factors together, HIV and hypoalbuminemia were significant and thus predisposing the patients to drug induced liver injury. Other factors were not statistically significant.

Subgroup analyses

Subgroup analyses were done on significant variables. For example, HIV positive patients are more predisposed to have extensive disease. So the question is, whether HIV infection is confounding the relationship between disseminated disease and occurrence of DILI. Hence the following subgroup analyses were done to understand HIV and disseminated TB as independent risk factors.

Table 25 HIV infection and disseminated disease

	Local (%)	**Disseminated (%)**	**OR**	**95 %C.I**
HIV Present	32(44.5)	40(55.5)	2.454	1.84-3.28
HIV Absent	246 (77)	72(23)	p-value 0.001	

The above chi-square table shows that HIV patients are predisposed to disseminated disease. 55.5% of HIV TB was disseminated compared to 23 % non-HIV TB (OR 2.454, p value 0.001, 95% C.I 1.84- 3.28). Hence subgroup analysis of relationship between extent of TB and DILI was examined in HIV negative patients. Even among HIV negative patients, disseminated disease predisposes to DILI 2.5 times more than localized disease (p value 0.019, OR 2.592, 95% CI 1.14 – 5.87).

Table 26 Extent and development of DILI among HIV negative patients

Extent	**DILI (%)**	**No DILI (%)**	**Odds ratio**	**C.I**
Local	16(59)	230(79)	2.592	1.14-5.87
Disseminated	11 (41)	61 (21)	p-value 0.019	

Similarly in patients with localized disease, presence of HIV infection was statistically associated with development of drug induced hepatitis (p value 0.016, OR 3.31 and 95% C.I 1.19-9.21).

Table 27 HIV infection and development of DILI among patients with localized disease

HIV	DILI (%)	No DILI (%)	Odds Ratio	95 %C.I
Yes	6(27)	26(10)	3.31	1.19-9.21
No	16 (73)	230 (90)	p-value 0.016	

HIV patients who have disseminated disease are mostly started on daily regimen. Hence the question was whether HIV infection is confounding the relationship between daily/thrice weekly and the occurrence of DILI. Hence both regimens were compared on HIV negative patients. Even among HIV negative patients, daily regimen was significantly predisposed more to ATT induced hepatitis compared to thrice weekly regimen(11% Vs 3%, p value – 0.014, OR 4.18, 95% CI 1.23- 14.24).

Table 28 Regimen and development of DILI among HIV negative patients

Regimen	DILI (%)	No DILI (%)	Odds Ratio	95 %C.I
Daily	24(11)	191(89)	4.18	1.23-14.24
DOTS	3 (3)	100(97)	p-value 0.014	

Patients who have disseminated disease are more frequently started on daily regimen. Hence the question was whether disseminated disease was confounding the relationship between daily/thrice weekly regimens and the occurrence of DILI. Therefore the relationship between daily/thrice weekly regimens and DILI were compared in patients with localized disease. Even in those patients, daily regimen was an independent risk factor of ATT induced hepatitis (p value 0.007, OR 4.8 and 95 % C.I 1.39-16.56)

Most of the patients on DOTS regimen were enrolled from secondary hospital of the Community health and department (CHAD hospital). The majority of TB in this setting was localized disease probably because CHAD is a secondary hospital. Therefore could referral bias, have influenced the relationship between localized disease and DILI. Hence subgroup analysis of patients with localized disease was done to see whether daily regimen was a independent risk factor for ATT induced hepatitis. Even then, daily regimen was a significant risk factor for ATT induced hepatitis (p value 0.007, OR 4.8 and 95% C.I 1.39-16.56).

Table 29 Regimen and development of DILI among patients with localized disease

Regimen	**DILI(%)**	**No DILI (%)**	**Odds Ratio**	**95 %C.I**
Daily	20(12)	150(88)	4.8	1.39-16.56
DOTS	3 (2.7)	108(97.3)	p-value 0.007	

From the different subgroup analysis that addresses the issue of confounding, we conclude that HIV infection, disseminated disease and daily regimen were independent risk factors for developing ATT induced hepatitis.

Predictive scoring system

We also propose a scoring system from our study so that patient at risk can be identified early before initation of treatment. The scoring system was derived based on the significant risk factors for predicting DILI. Scores were given according to the odds ratio of the significant risk factors in the multivariate logistic regression analysis. HIV infection, chronic liver disease, daily treatment regimen, extent of tuberculosis, hypoalbuminemia and female gender are the risk factors included in the scoring system. The risk factors along with their scores are summarized as below.

Table 30 Predictive scoring system based on risk factors for DILI

Risk factors for DILI	**Score**
HIV infection	3
Chronic liver disease	4
Daily treatment Regimen	2
Extent of tuberculosis(disseminated)	2
Hypoalbuminemia (S.albumin < 3.5 g/dl)	2
Female gender	2

Significance of the scoring system was calculated using Receiver Operator Characteristics(ROC) curve.

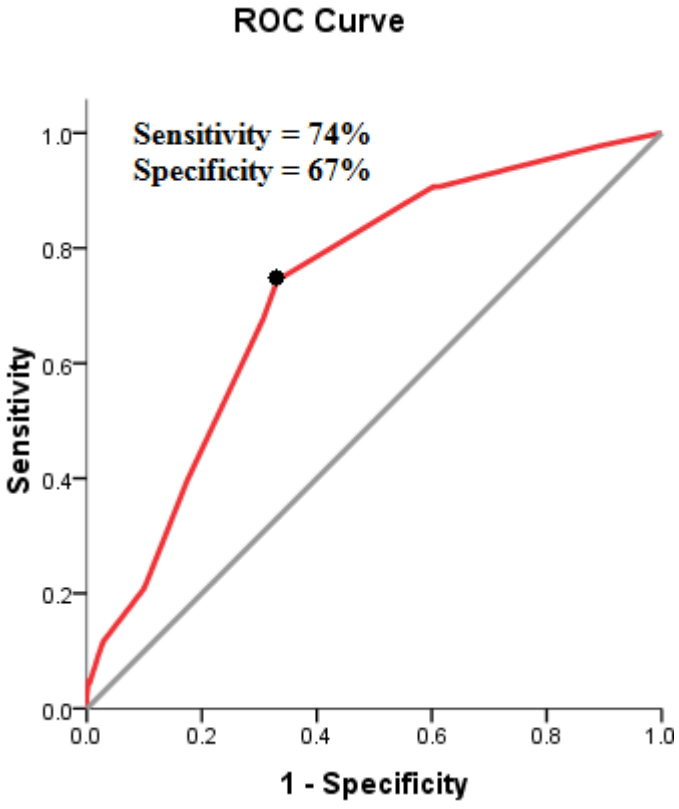

Fig.13 ROC curve for the predictive scoring system

Area under the curve – 0.728

Standard error – 0.037

Confidence interval – 0.65 to 0.80

Table 31 Summary of sensitivity and specificity for predictive score

Score	**Sensitivity**	**Specificity**
2.5	0.907	0.386
4.5	0.744	0.669
5.5	0.674	0.694
6.5	0.395	0.826
8.5	0.209	0.900
10.5	0.093	0.980
12.5	0.047	0.997

The area under the curve for the above mentioned scoring system was calculated to be 0.728 which indicates this predicve score is a fairly good score for predicting DILI. The sensitivity and specificity for individual scores were calculated be plotting the area under the curve as mentioned above in table 31. A total score of 5 and above predicts DILI with the sensitivity of 74% and specificity of 67%. However this scoring system has to be validated by the prospective studies.

Clinical Profile of patients with drug induced liver injury (DILI)

Out of 43 patients who developed drug induced liver injury, 20 patients were males. 24 patients had disseminated disease whereas 19 patients had local disease.

Among 43 patients, 39 patients were on daily ATT and only 4 patients on intermittent thrice weekly DOTS regimen.

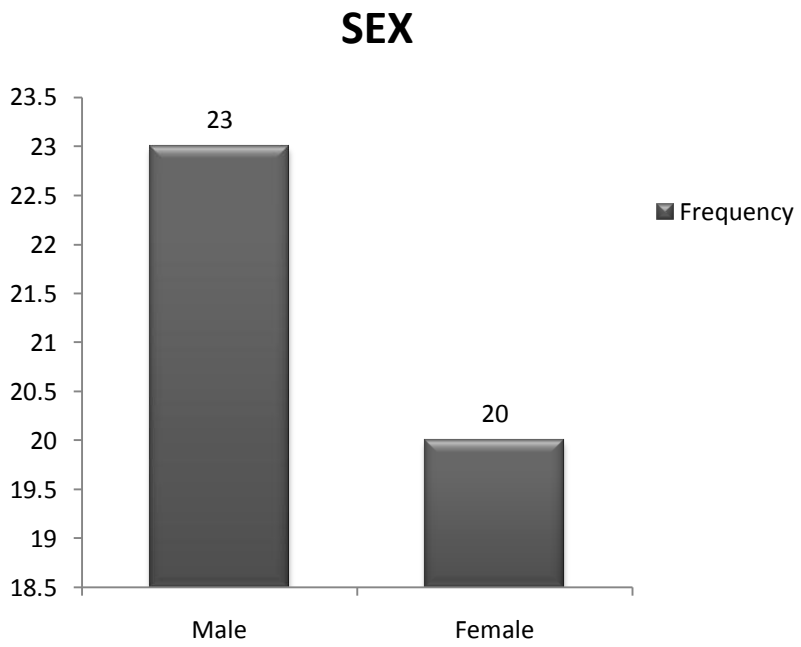

Fig.14 : Gender distribution of the patients with ATT induced hepatitis

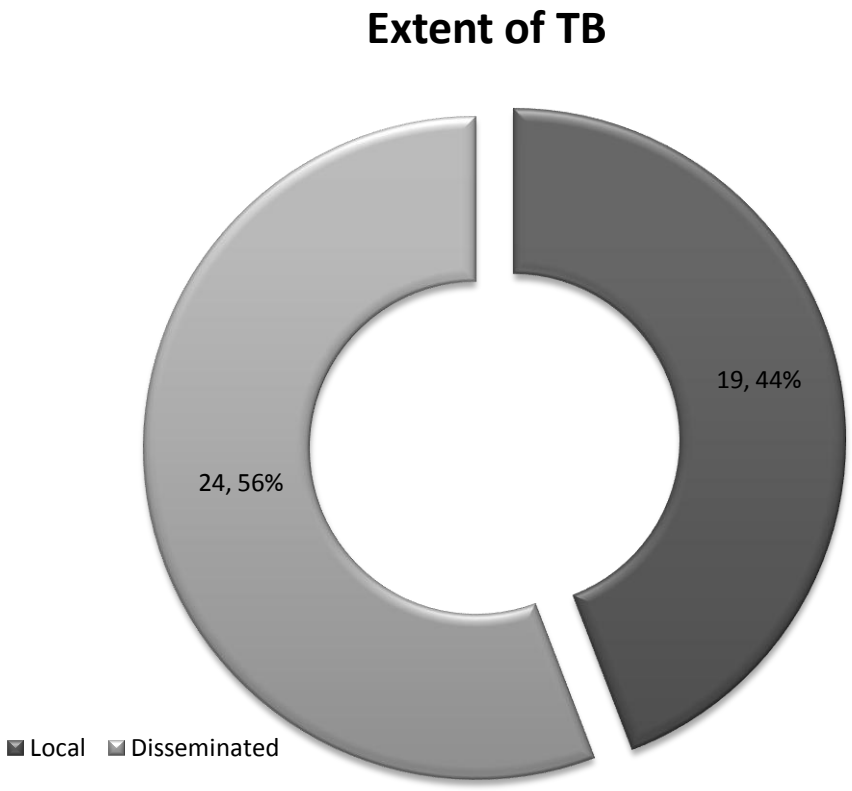

Fig 15: Extent of tuberculosis among patients with ATT induced hepatitis.

Clinical Features

Vomiting was the most common symptom seen in 58.1% of patients of patients with drug induced hepatitis followed by jaundice in 30.2 % of patients. Other symptoms in the order of frequency were fever, pruritis, nausea and abdominal pain. However 7 patients (16.3%) were asymptomatic and diagnosed based on enzyme elevation more than 5 times.

Table 32: Symptoms profile of patients with DILI

Symptoms	Frequency	Percentage (%)
Fever	6	14
Vomiting	25	58.1
Abdominal pain	2	4.7
Jaundice	13	30.2
Pruritis	3	7
Nausea	2	4.7
Dyspnea	1	2.3
Asymptomatic	7	16.3

On examination, icterus was observed in 28 patients (65%) followed by hepatomegaly in 2 patients. Signs of decompensation were seen in 4 patients. Other patients did not have any findings on examination.

Table33 Signs of Hepatic Decompensation in patients with DILI

	Number	Percentage
Ascites	2	4.7
Encephalopathy	3	7.0

Current Regimen

Patients who developed drug induced liver injury were on the following regimen before they developed hepatotoxicity

- **Isoniazid, Rifampicin, Pyrazinamide and Ehtambutol (HRZE)** in 41 patients(95.3%)

- **Isoniazid, Rifampicin, Pyrazinamide and Ehtambutol and Streptomycin(HRZES)** in 1 patient (2.3%)
- **MDR regimen** in 1 patient (2.3%)

As presented earlier 90.7% of patients with DILI were on daily TB treatment regimen (39/43)

Alternate regimen was started after development of DILI as follows:

- ✓ **Amikacin, Levofloxacin and Ethambutol (ALE**)in 30 out of 43 patients
- ✓ **Isoniazid, Pyrazinamide, Ethambutol and Levofloxacin (HZEL)** in 4 patients. This was started in patients with drug induced cholestasis probably due to Rifampicin.

Onset of DILI after initiation of anti-tubercular drugs

Drug induced liver injury was seen as early as first day till 153 days after initiation of anti-tubercular drugs. Majority of patients (77%) developed drug induced liver injury within first 2 months. 20 patients developed DILI within 2 weeks followed by 13 patients from 2 weeks to 2 months.

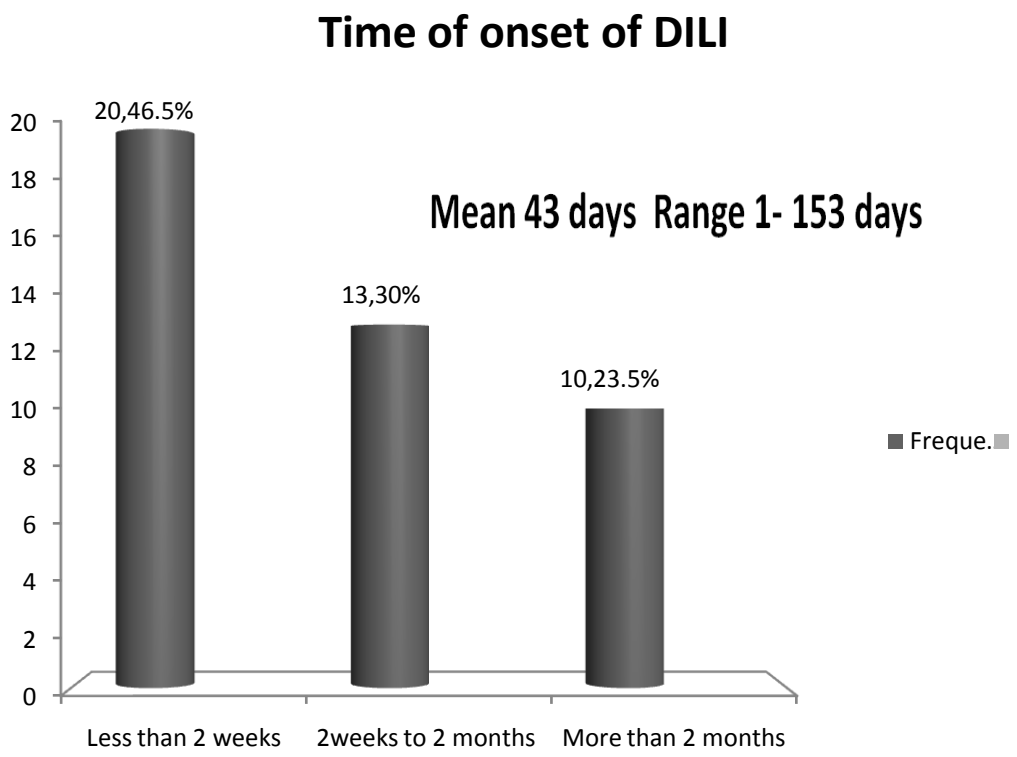

Fig.16 : Time to onset of ATT induced hepatitis

The mean time duration for normalization of liver function was 22 days ranging from 3 to 81 days.

Type of hepatitis and severity

Type of hepatitis was classified as cholestatic, icteric and anicteric hepatitis. Icteric hepatitis was the most common type seen in 26 patients (60%) followed by cholestatic pattern in 11 (26%) and anicteric hepatitis in 6 patients (14%) and the order of frequency.

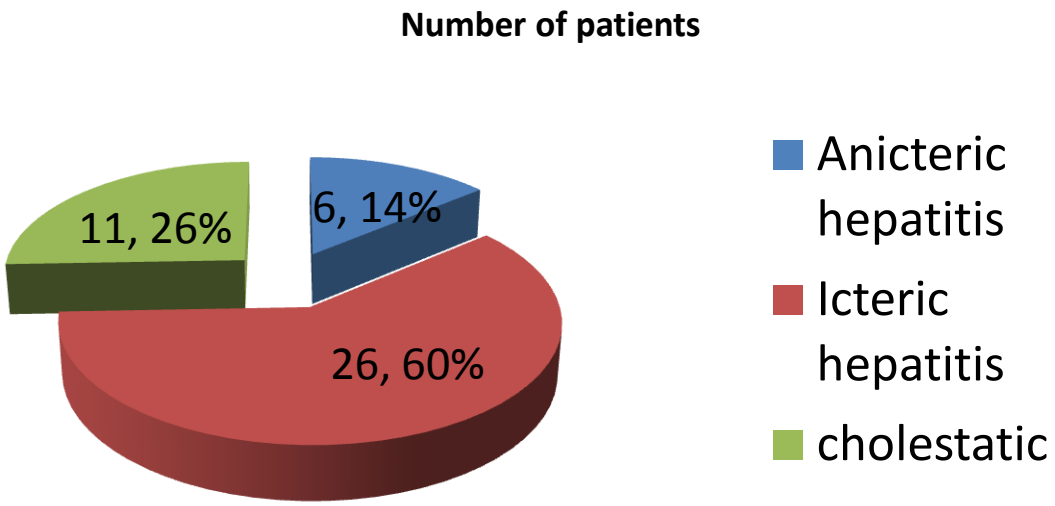

Fig. 17 Types of hepatitis in patients with DILI

Severity of hepatitis was according to WHO toxicity standards into mild, moderate and severe hepatitis. 15 patients (35%) had severe hepatitis. 13 patients (30%) had moderate hepatitis and 12 patients (28%) had mild hepatitis.

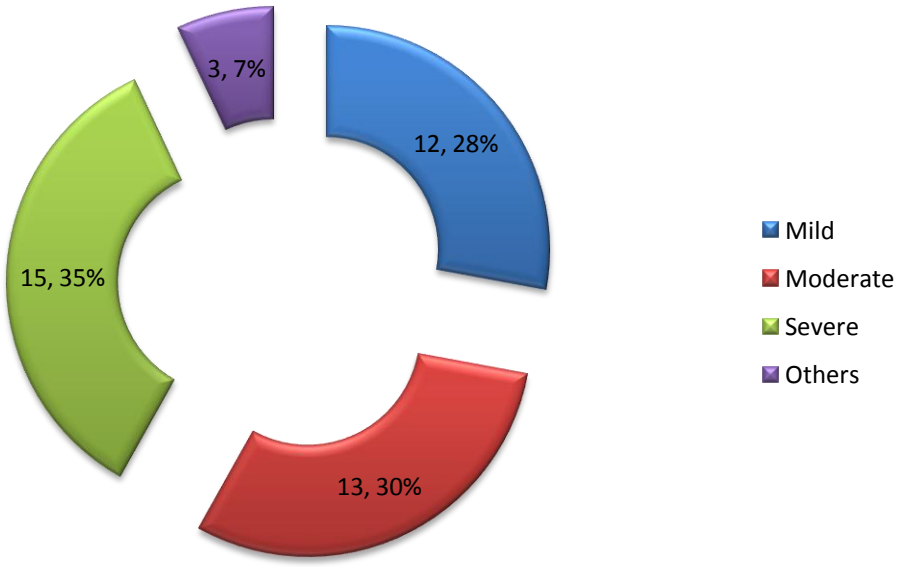

Fig. 18 Severity of hepatitis

Outcome of patients with drug induced liver injury

Forty one patients were regular on treatment till the developed hepatotoxicity. Hospitalization was required for 39 patients(90.7%).Four of them needed ICU care, out of which 3 of them improved and one died. 36 patients had complete resolution(84%). Four patients had features of acute hepatic failure out of which 3 patients recovered. All cause mortality was 4.7 % (2 patients). The causes of death were acute liver failure in one patient and pulmonary embolism in the other patient.

Rechallenge of anti-tubercular drugs

There are three main approaches to reintroduction of first line drugs.

- ✓ Reintroduction of full dose of one drug at a time preferably Rifampicin followed by Isonizaid and Pyrazinamide (according to ATS guidelines).
- ✓ Reintroduction of escalating doses of one drug at a time (according to British Thoracic Society guidelines).
- ✓ All three drugs at a time (full doses)

Among 29 patients who were rechallenged, at least 1 drug was successfully rechallenged in 28 patients. All 3 hepatotoxic drugs were reintroduced successfully in full doses at the same time in 2 patients. Both of them had mild hepatitis. Rechallenge by both ATS and BTS guidelines had similar successful rate. Rechallenge methods and their frequency were as follows:

- ❖ One drug at a time in full dose (ATS guideline) -58.6%
- ❖ One drug at a time in escalating dose (BTS guideline)-34.5%
- ❖ All drugs-6.9%

Isoniazid was the first drug rechallenged in 23 out of 31 patients which was followed by Rifampicin in 8 patients. Rifampicin was not rechallenged in 2 patients in view of cholestatic

picture most probably due to Rifampicin. Hence Levofloaxin was started on those 2 patients.

Successful Rechallenge

All three drugs are succesully rechallenged in 5 patients. At least two drugs were rechallenged in 16 patients. Isoniazid was successfully rechallenged in 24 out of 28 patients and Rifampicin in 26 out of 29 patients. Pyrazinamide successfully rechallenged in all 6 patients

Rechallenge hepatitis to isoniazid and rifampicin developed in four and three patients respectively.

Table 34 Various guidelines and rechallenge hepatitis

	Rechallenge hepatitis		
	Isoniazid	**Rifampicin**	**Total rechallenge hepatitis**
ATS guidelines	2 out of 16 (12.5%)	2 out of 14 (14.2%)	4/30 (13.3 %)
BTS guidelines	2 out of 11 (18.2%)	1 out of 12 (8.3%)	3/23 (13 %)
ALL three drugs at a time	None	None	None

There was no apparent difference in the rates of rechallenge hepatitis between the two rechallenge regimens. Outcome of tuberculosis in patients with DILI

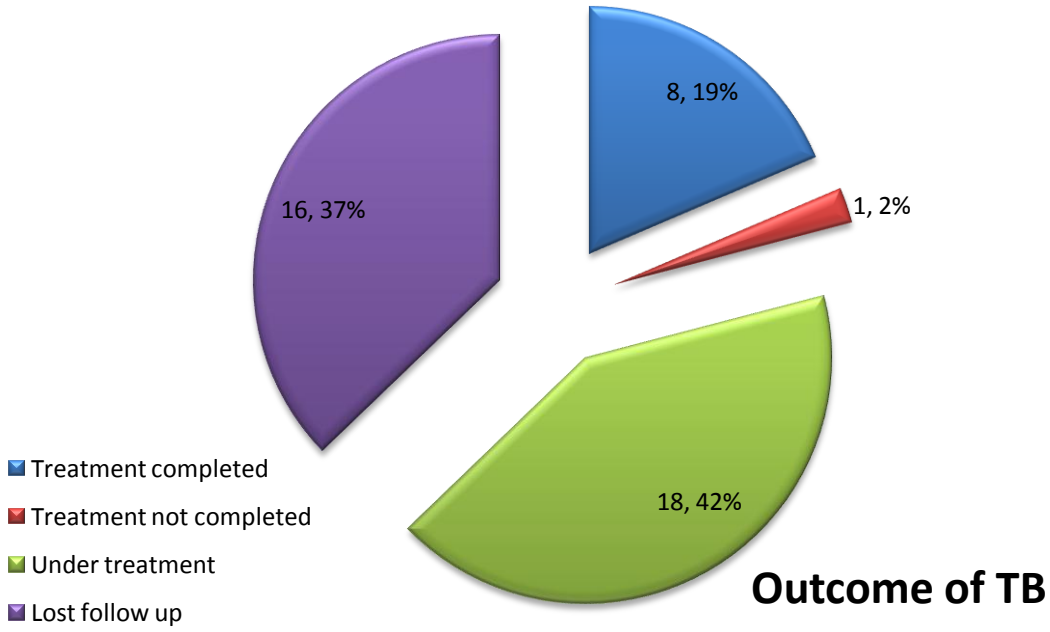

Fig. 19 Outcome of tuberculosis in patients with DILI

8 patients with DILI had completed their treatment and were cured of tuberculosis. One of the patients who expired did not complete treatment. 18 patients are under treatment and 16 patients had lost follow up.

Discussion

Tuberculosis remains as a major threat to developing countries like India despite availability of effective treatment which can cure most of the cases. India and China account for 40 % of world's total TB cases(2). One of the major reasons for discontinuing treatment is due to adverse reactions mainly drug induced liver injury (DILI). Anti-tubercular drug induced liver injury not only leads to interruption of treatment but also increases the risk of developing multi drug resistant and extremely drug resistant tuberculosis. However early identification of drug induced liver injury, starting the patients on alternate non hepatotoxic regimen and subsequent rechallenge if possible can prevent the above mentioned risks and decrease morbidity and mortality related to DILI. It is this background that this study was conducted to determine the incidence, risk factors, clinical course and outcomes of anti-TB drug induced liver injury. The overall goal of the study was (a) towards developing an appropriate screening strategy to predict DILI and (b) appropriate management strategy for DILI to improve outcomes of both liver injury and tuberculosis.

Baseline characteristics

In our cohort, majority of the patients were male (61%) and in the middle age group (81%). 45% had normal body mass index but a significant number of people (34%) were underweight. Few recent studies from India showed increased prevalence of tuberculosis among diabetics. In our cohort, diabetes was present in 35% of the patients of age more than 35 years. Mean HbA1c was 8.75 suggesting that uncontrolled diabetics were more predisposed to chronic infections like tuberculosis. However more studies are needed to support this information. If so, there is a threat for increase in number of new patients diagnosed with tuberculosis due to increasing prevalence of diabetes mellitus in India. HIV infection was seen in 72 patients (18.3%) which is higher than reported in other Indian studies(54).

Incidence of drug induced liver injury

Incidence of drug induced liver injury in our study was 9.7% (CI 7-13.2%). The large study and narrow confidence intervals suggest that the incidence is fairly accurate. The incidence varies between different countries with lower incidence in western countries compared to developing countries like India. This study confirms the higher incidence of DILI in TB in our country.

A meta analysis of 14 published studies (3) from western countries showed an incidence of 4.38%. Incidence of ATT induced hepatotoxicity from our study was comparable to the studies from Asian countries. A Malaysian study published in Singapore Medical Journal by Marzuki et al showed an incidence of 9.7% (95% C.I 7-13.2%) similar to our study(57). Incidence of ATT induced hepatitis was 16% according to a North Indian study by Sharma et al(16) and 10.5% according to a study by Deepak et al(54). The reason for higher incidence of ATT induced hepatitis in Asian countries is clearly unknown. Ethnic susceptibility and genetic polymorphisms may play a role in increased incidence of hepatotoxicity. While this may be the case, in the risk factor analysis we have attempted to explore the role of common acquired risk factors such as under nutrition, extensive TB and HIV infection in the development of DILI in our setting.

In our study, 279 patients were on daily regimen and 114 patients were on intermittent thrice weekly DOTS regimen. Incidence of ATT induced hepatitis was 14% (95% C.I 7.9% - 22.4%) among daily regimen group as compared to 3.5% (95% C.I 2.4% - 4.8%)in DOTS regimen group. This study confirms the lower incidence of DILI in DOTS that has been reported in earlier studies. The lower incidence in DOTS regimen group was similar to a Chinese study by Shang et al(4). In that prospective study of 4304 patients who were on DOTS regimen, incidence of drug induced liver injury was 2.55%. The majority of the patients who were on DOTS treatment were receiving treatment in the community hospital,

had localized TB disease and lower rates of HIV infection. These too may have contributed to the lower incidence of DILI on DOTS. Another study from Tirupathi(58) showed a DILI incidence of 3.3% with DOTS. However another study from Hong Kong(59) did not confirmed this finding. This could have also been due to the high background rates of hepatitis B carrier status.

Model of impact of DILI on TB treatment based on our study

If 400 patients diagnosed with tuberculosis are started on TB treatment the following are the impacts of DILI

- 40 patients develop DILI
- Nearly all DILI patients require hospital admission
- 4 patients will develop acute liver failure
- 1 patient will die of DILI
- Nearly all patients will require modification of TB regimen
- The impact on treatment failure, relapse and drug resistance is not known

The significance of DILI is brought out in the above box which shows that if four hundred patients were diagnosed with tuberculosis, DILI develops in forty patients. Four patients will develop acute liver failure and and one patient will die due to DILI. This model helps us understand the impact of DILI on TB treatment.

All these indicate that significant effect on patients lives and public health importance of DILI on morbidity and mortality of tuberculosis.

Predictors of liver injury

Bivariate analysis in this study showed that hypoalbuminemia , HIV infection, underlying chronic liver disease, disseminated disease and daily regimen were significantly associated with the development of DILI. These findings are consistent to other published studies and confirm these findings.

HIV and disseminated disease

Among the significant risk factors, disseminated disease was significantly associated with developed DILI with odds ratio of 1.769 and p value 0.006. Forty seven percent of patients in the DILI group had disseminated disease when compared to 26% of those who did not develop DILI . Other significant risk factor was the presence of HIV infection. 36% of patients who developed DILI were HIV positive compared with 16% of patients with HIV infection in those who did not develop DILI with odds ratio of 2.84 and p value of 0.002. In our cohort, HIV patients were more predisposed to disseminated disease (p value 0.006) which is already known(60)(61,62). Hence further subgroup analyses were done to understand whether either factor was confounding the relationship of other factor to the occurrence of DILI. HIV infection was still significant even in patients with localized disease (p value 0.016, OR 3.31). Also disseminated disease was a significant risk factor for development of DILI even in HIV negative patients (p value 0.019, OR 2.59). Hence we can conclude that both HIV infection and disseminated disease were independent risk factors for development of DILI.

Chronic liver disease

Presence of chronic liver disease was a significant risk factor for development of DILI (p value 0.004, OR 4.72). However there were only 14 patients with underlying chronic liver disease in our study. Hence while interpreting these conclusions, the limitation of the smaller number should be kept in mind. This findings is consistent with the literature regarding the

higher rates of hepatoxicity in underlying chronic liver disease (21,22). In view of the high risk of DILI with underlying chronic liver disease, we recommend that these patients may be started on non-hepatotoxic regimen initially and subsequently challenged with first line hepatotoxic drugs one at a time.

Hypoalbuminemia

The other significant predictor of liver injury was hypoalbuminemia (p value 0.045, OR 1.92). However this may be a confounding factor since other significant factors like HIV infection, disseminated disease and underlying chronic liver disease are known to cause hypoalbuminemia. This study confirms the earlier findings that hypoalbuminemia is a risk factor for DILI (29)(16).

DOTS versus daily regimen

The single most risk factor after chronic liver disease for development of DILI from our study was daily regimen (p value 0.003, OR 4.469). Fourteen percent (95% C.I 2.4%-4.8%) of patients on daily regimen developed DILI when compared to 3.5% (95% C.I 7.9% - 22.4%) patients on DOTS regimen. This is the only significant modifiable risk factor. Patients at higher risk of DILI can be potentially shifted to thrice daily DOTS regimen so as to reduce the risk of DILI. We also did subgroup analyses among HIV negative patients and those with localized disease. Even among HIV negative patients daily regimen was a significant risk factor for development of DILI.

The reason why DOTS regimen had lower rates of hepatotoxicity despite similar doses per kilogram body weight is unknown. The difference between pharmacokinetics of daily therapy and DOTS in relation to liver, is the sustained higher level of drug concentrations in daily therapy. Therefore the toxicity appears to be time dependent (adequate concentration sustained over time) rather than concentration dependent. Similar

differences are seen in aminoglycosides induced nephrotoxicity with divided daily dose compared to single daily dose(63).

In an Indian study (31) published by Mandal et al in 2012, administration of daily regimen predisposed the patients more to drug induced liver injury as compared to intermittent DOTS regimen (7.5% versus 2.32 %). This study included only patients with sputum positive pulmonary tuberculosis and was followed only till completion of intensive phase. Both regimens had equal sputum conversion rate at the end of intensive phase. However default rate was more in the DOTS group (9.3% versus 5%). Given the rates of DILI in both regimen groups, it is clear that DOTS regimen is associated with lower rates of hepatotoxicity.

From our study, DOTS regimen was be considered as a treatment option in patients with high risk of developing DILI. However in our study we have not yet obtained data on outcomes of tuberculosis including relapse and drug resistance on long term follow up of our patients. WHO is currently advocating daily treatment regimen for tuberculosis because of more rapid sterilization (culture negativity) and lower rates of relapse(64). However the treatment trials comparing the daily and DOTS regimen as well as the clinical care guidelines have underemphasized the importance of the drug toxicity of DILI. Our study shows that patients on daily regimen have a 4.5 times greater risk of DILI requiring treatment modification, risk of acute hepatic failure and death. The impact of DILI on the overall clinical outcomes of tuberculosis is as a yet unclear.

Model of impact of intermittent DOTS on DILI based on this study.

If 400 patients diagnosed with tuberculosis and started on DOTS treatment,

14 patients will develop DILI

None will develop acute liver failure

No deaths will occur due to DILI

The comparative impact on TB cure, relapse and drug resistance is unclear

"26 CASES OF DILI WILL BE PREVENTED AMONG 400 PATIENTS STARTED ON DOTS IN COMPARISON TO DAILY REGIMNE"

Our study shows that if 400 patients are started on TB treatment, we can prevent 26 cases of DILI with DOTS therapy as seen above. However the impact of this strategy on treatment efficacy and relapse rates needs further study. It appears that daily treatment regimens are a double edged sword. They have faster sterilizing rate, lower relapse and drug resistance but higher hepatoxicity. On the other hand intermittent DOTS regimen are better tolerated but have relatively worse TB outcomes. Therefore we suggest the reevaluation of DILI on the impact of TB treatment and potential benefit of DOTS in reducing DILI.

Non significant risk factors

Other factors such as gender, age and body mass index reported in earlier studies were not significantly associated with development of DILI. In the study published by Singla et al, significant risk factors for development of DILI included age more than 35 years and mid arm circumference < 20 cm. In another study by Pande et al, one of the risk factors associated with development of DILI was older age. In another study which was recently published by Pore et al, female gender was significantly associated with development of DILI. In the multivariate analysis of our study, female gender on analysis showed a p value of 0.09 but the

Odds ratio was of 1.97 indicating that it may be a significant risk factor for predicting DILI. There might be a gender predisposition to develop DILI but this was definitely overweighed by other risk factors. Cytochrome P450 (CYP) 3A4 is expressed at higher levels in women which can result in more toxic intermediates. A prospective study from Taiwan (2014) compared 355 TB patients with 475 healthy subjects(65). Eight single nucleotide polymorphisms of PXR gene were performed since PR gene controls CYP3A4 expression. This study showed PXR influences the susceptibility to tuberculosis with the increased risk among females..

Mutivariate logistic regression analysis

Multivariate logistic regression analysis was done based on three different models as described above in the results section. Analyzing 3 models separately helped us understand the influence of interacting risk factors for development of drug induced liver injury. Chronic liver disease, hypoalbuminemia, HIV infection and daily regimen were the independent risk factors for developing drug induced liver injury. Disseminated disease was already known as a significant risk factor and similarly found in our bivariate analysis for development of DILI (p value 0.006). The limited sample size and the interactions between disseminated TB, HIV infection and hypoalbuminemia may have diminished the influence of disseminated TB in the logistic regression analysis. Hence disseminated disease cannot be excluded from the list of significant risk factors. Thus we suggest careful monitoring of patients started on anti-tubercular drugs in the presence of chronic liver disease, hypoalbuminemia, HIV infection, disseminated TB and patients who are on daily TB treatment regimens.

The genetic predisposition of the patients to drug induced liver injury is well known based on recent studies(38,45,48). The clinical predictors of DILI in our study emphasized the role of acquired risk factors (HIV infection, disseminated tuberculosis and low albumin).

What we observed from our study was that the combination of acquired risk factors predisposed the patients to DILI rather than a single risk factor. In practice this means that a patient who has disseminated TB and HIV or disseminated TB with undernutrition is at substantially higher risk of developing DILI. It does not seem to be an individual risk factor, but rather a combination of risk factors that renders the liver vulnerable to injury. The reason why these risk factors such as HIV infection, hypoalbuminemia, disseminated disease in combination predisposes to hepatotoxicity even in the absence of underlying liver disease is not clear. We suggest that the combination of these risk factors may lead to either : a)increased vulnerability of hepatocytes to normal TB drug metabolite levels or b) altered metabolism of TB drugs leading to higher concentration of hepatoxic metabolites. This hypothesis is consistent with clinical experience that patients can tolerate full rechallenge of all hepatotoxic drugs once their general clinical condition has improved. In summary, there appears to be an inherent succeptibility of the state of advanced tuberculosis to the development of DILI, the mechanism of which is at yet unclear.

Predictive scoring for development of DILI

According to the predictive score for development of DILI as described above, the sensitivity and specificity for the score of 5 and above was 74% and 67% respectively.

Table 34 Scoring system

Risk factors for DILI	**Score**
HIV infection	3
Chronic liver disease	4
Daily treatment Regimen	2
Extent of tuberculosis	2
Hypoalbuminemia (S.albumin < 3.5 g/dl)	2
Disseminated disease	2

Total Score of ≥ 5 predicts DILI with sensitivity of 74% and specificity of 67%

Based on the above mentioned scoring system, DILI can be predicted with a sensitivity of 74% (ie. Three out of 4 patients who will develop DILI can be identified before starting treatment). According to the scoring system, if the patient has HIV infection or chronic liver disease in combination of any one risk factor (hypoalbuminemia, disseminated disease, daily regimen and female gender), there is high risk for development of DILI. In the absence of HIV infection and chronic liver disease, a combination of 3 other risk factors (hypoalbuminemia, disseminated disease, daily regimen and female gender), increases the risk of DILI. Therefore in the presence of chronic liver disease, those patients can be started on non-hepatotoxic regimen initially and subsequently challenged with first line hepatotoxic drugs one at a time.

Based on the above scoring system we propose the following clinical rule for prediction of DILI before starting TB treatment.

Proposed Clinical rule for prediction of DILI (based on scoring system)

Situations of high risk of DILI

1. Chronic liver disease + any one risk factor (low albumin or HIV infection or disseminated disease or female gender).
2. HIV + any one risk factor (low albumin or chronic liver disease or disseminated disease or female gender).
3. Low albumin+ Disseminated disease+ female gender (In patients who are HIV negative and no chronic liver disease).

In all above mentioned situations initiation of low toxicity DOTS regimen can be considered

Although ATT induced hepatotoxicity is a major public health problem, there is no prediction rule for clinical practice. We suggest that the application of this scoring system before initiating TB treatment can reduce the risk of DILI. This prediction rule requires validation in further clinical studies.

Profile of patients who developed DILI

43 patients developed DILI out of which 23(53%) patients were male and 24(45%) had disseminated disease. One patient out of 43 patients developed DILI was on MDR regimen who was successfully rechallenged after withdrawing the pyrazinamide, the most probable culprit drug. Most of the patients (76.7%) developed DILI within first 2 months with 53% of patients developing DILI within first 2 weeks. Thus we suggest careful clinical and biochemical monitoring during first 2 months in patients with clinical risk factors. However 10 out of 43 patients developed DILI even after 2 months.

Vomiting and jaundice are the common symptoms observed in those patients. 16% of patients were asymptomatic and diagnosed was based on deranged liver function tests. Those patients developed DILI even after 2 months which suggests that this reflects true drug

induced liver injury rather than hepatic adaptation. The most common type of hepatitis was icteric hepatitis seen in 26 patients(60%). 15 patients (35%) had severe hepatitis with enzyme elevation more than 400 IU. We also did subgroup analysis on patients who developed severe hepatitis. None of the factors analyzed except underlying chronic liver disease were found to be significant risk factor for developing severe hepatitis. Most of had complete resolution of liver functions after withdrawal of offending drugs. Low mortality rate (4.3%) was seen among patients who developed DILI. Acute liver failure was seen in 4 patients with one patient succumbing to the illness. Hence early identification can decrease morbidity and mortality associated with DILI.

Rechallenge of anti-tubercular drugs

Isoniazid was the first drug rechallenged followed by rifampicin in most of the patients. Pyrazinamide was rechallenged only in 6 patients which was successful in all patients. Hence we suggest that whenever possible, rechallenging the patients with pyrazinamide may be attempted at escalating doses according to British Thoracic Society guidelines. Among various rechallenge methods, introducing one drug at a time in full dose according to American thoracic society guidelines was observed in 58.6% of patients. Among 29 patients who were rechallenged, atleast 1 drug was successfully rechallenged in 28 patients (96.5%). Two patients were rechallenged with all 3 drugs at a time in full doses. Both of them had mild DILI and did not develop rechallenge hepatitis. Hence we suggest rechallenge of all 3 drugs can be attempted in patients with mild hepatitis in absence of clinically significant risk factors.

A recent study published by Zuberi et al from Karachi(66) showed no significant difference between ATS and BTS guidelines(p value <0.7). However ATS guideline was easier to follow. Another study from AIIMS(53) compared three different methods of ATT drugs reintroduction which showed no significant difference between the three groups.

Rechallenge hepatitis to Isoniazid and Rifampicin was seen in four and three patients respectively. Different methods of rechallenge were compared with rechallenge hepatitis (Refer table 34). The rates of rechallenge hepatitis were similar in patients who were rechallenged according to both ATS and BTS guidelines(13.3% Vs 13%). Our results are consistent with the published literature that there is a lack of difference in risk of rechallenge hepatitis between the two rechallenge regimens.

Our suggestions regarding rechallenge regimens based on these results are:

- Rechallenge according to ATS guidelines is safe and acceptable. Patients with chronic liver disease should be rechallenged with escalating drug doses according to BTS schedule with minimum number of hepatotoxic drugs.
- Patients with asymptomatic DILI can probably be rechallenged with all three hepatotoxic drugs including Pyrazinamide. However we suggest that rechallenge with Pyrazinamide can be done with escalating drug doses.

Limitations for our study

Our study was a hospital based prospective study from a tertiary hospital in South India. We studied on incidence, clinical predictors of DILI, profile of patients with DILI, outcome of hepatitis and tuberculosis including rechallenge of first line drugs. Very few prospective studies have been done before from India with such a sample size covering all these aspects. Our study has few limitations. We have not completed our sample size and we are planning to continue this study. Another limitation is the significant number of patients (32%) who had lost to follow up. We are planning to follow up those patients through telephone subsequently.

Future work and research

The predictive scoring system proposed from our study needs to be validated by a well designed prospective study. Further follow up of patients with DILI is being planned to assess relapse, default rate and drug resistance. Genetic predisposition also seems to play a role in drug induced liver injury apart from clinical risk factors and the role of NAT2 polymorphisms in the Indian setting needs further study. More studies are required in this area so that we can derive a score based on both clinical and genetic risk factors that can predict DILI before initiation of treatment. If the state of disseminated TB increases vulnerability of the liver to drug induced hepatitis, the role of nutritional and other hepatoprotective regimens in reducing the risk of DILI needs further study.

Conclusions

- Incidence of anti-tubercular drug induced hepatitis from our study was 9.7% (95% C.I 7-13.2%). The high incidence of DILI associated with TB treatment emphasizes the public health importance of this side effect in India. Intermittent DOTS regimen was associated with a DILI rate of 3.5% (95% C.I 2.4%-4.8%) compared to the rate of 14% (95% C.I 7.9-22.4%) with daily treatment regimen. This confirms a substantially lower rate of DILI in DOTS treatment compared to daily TB treatment regimen.
- HIV infection (OR 2.84, p value 0.002, 95% C.I 1.42 – 5.67), daily regimen (OR 4.46. p value 0.003, 95% C.I 1.55 – 12.81), disseminated disease (OR 1.769, p value 0.006, 95% C.I 1.23-2.55), hypoalbuminemia (OR 1.92, p value 0.045, 95% C.I 1.01 – 3.68) and chronic liver disease (OR 4.72, pvalue 0.004, 95% C.I 1.5-14.82) were independent risk factors for development of drug induced liver injury. The results emphasize the importance of acquired risk factors in development of drug induced liver injury. A prediction score based on the above risk factors is suggested to identify patients who will develop DILI. A score of $\geq$ 5 predicts DILI with a sensitivity and specificity of 74% and 67%. The application of this score in clinical practice can reduce risk of DILI. In patients with high risk of DILI identified by the scoring system, DOTS thrice weekly regimen can provide an alternate treatment strategy to reduce DILI risk. Further work is needed to validate the predictive scoring system derived from our study.
- Mortality rate seems to be low (4.3%) among patients who developed DILI. Four patients (9.3%) developed acute liver failure. Most patients (84%) of patients had complete resolution after discontinuation of offending drugs.
- At least one drug was rechallenged successfully in 96.5% patients. Rechallenge hepatitis to isoniazid and rifampicin developed in 4(14%) and 3 patients (10%) respectively.

- Rechallenge by both ATS and BTS guidelines had similar successful rate.

  Rechallenge according to ATS guidelines with full dose of one drug at a time may be recommended for most cases of DILI. However British thoracic guideline for rechallenge with escalating dose of each drug sequentially administered, may be preferred in those with chronic liver disease and for pyrazinamide rechallenge.

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APPENDIX

APPENDIX I: INFORMED CONSENT DOCUMENT

Date:

I …….(Participant's name), Hosp no…… have fully read and understood the participant's information sheet as given above.

By signing this form I agree that

(1) I understand that the purpose of this study is to improve the quality of medical care and that my involvement may not benefit me.

(2) I have been made aware of the procedures involved in the study and the expected inconvenience, risk, discomfort or potential side effects as far as they are currently known by the researcher.

(3) My participation in this study is fully voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

(4) I understand that my identity will not be revealed in any information released to third parties or published.

(5) I will not restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s)

I do hereby agree to take part in this study.

Patient Name Signature Date

(or Thumb impression) of the Subject/Legally Acceptable Representative

Investigator Name Signature Date

Witness Name Signature Date

APPENDIX II: PATIENT INFORMATION SHEET

1. Study title

PREDICTORS, OUTCOME, PROFILE OF ANTI-TUBERCULOSIS DRUG INDUCED HEPATITIS – A prospective nested case- control study in a South Indian tertiary hospital.

2. Principal Investigator Dr. Selvin Sundar Raj M

3. Contact address

Department of General Medicine Uni1,

Christian Medical College, Vellore 632 004, India

Email: selvinsr@gmail.com

Tel. + 91 416 2282089

This study is a research project conducted in CMC Vellore, Outpatients and In-patients of Department of General Medicine and DOTS clinic (CHAD), CMC Vellore. You know that you have been diagnosed to have tuberculosis and started on treatment for the same. A proportion of patients say around 12 % develop jaundice as a complication of TB treatment. This study aims to study the **clinical and genetic risk factors for TB drugs related jaundice.**

If you decide to participate in the study, you will be asked to give around 10 ml of blood collected from a vein near your elbow. This is the only invasive procedure that you will be subjected to.

All precautions necessary will be taken to avoid any complications that may arise due to the venipuncture. Your arm will be cleaned with spirit and left to dry. Puncture will be made using a needle attached to a disposal syringe.Compression will be applied for 2 minutes to

ensure that bleeding has stopped. This is all done as a one time process. Your treatment will not be altered based on any of the study results.

By participating in the study you will not be made to incur any added expenses. There is no added risk of any kind for you by participating in this study. The information generated by this study may not directly benefit you at this time but may benefit the other patients in future. Any personal information about you that is collected as part of this study will be maintained strictly confidential.

If you have any queries or problems you can contact the principal investigator at the above address. If you choose not to participate, it will not affect your treatment in any way.

APPENDIX III: Patient Enrollment Form

Consent taken: Yes/ No **Patient sent to VCTC:** Yes/No

Name: **Age:** **Sex:** **Study no:**

Contact No: 1) 2)

Address:

Hospital No:

Diagnosis:

1)Sputum +ve Pulmonary 2)Sputum negative pulmonary 3)Pleural effusion 4) LN 5)TBM 6)TB peritonitis 7) Disseminated 8)TB spine 9)Miliary TB 10)GUTB 11) CNS TB(tuberculomas) 12) TB OM 13) synovitis 14)other

Type of TB and extent:

Basis of diagnosis of TB:

1) Clinical 2) Radiological 3)Microbiological 4) Histopathological 5)Lab

Date of diagnosis: **Treatment started on**:

Regimen

1) Daily 2) Category I 3) Category II

Drug doses: **Drug frequency** 1) Daily 2) thrice daily

Risk factors:

1) Site of TB

   1) Pulmonary 2) Pleural effusion 3) lymphadenitis 4) TB meningitis 5) TB peritonitis 6) Disseminated

2) Extent of TB: 1) Local 2) Disseminated

3) BMI : Height : Weight: :

4) Underlying liver disease (details):1) alcoholic 2) hepatitis B 3)hepatitis C 4) None

5) Past history of jaundice : 1) Yes 2) No

6) Hypoalbuminemia :

7) Alcoholic: 1)Never 2) Past 3) Current

   Amount Frequency Duration (Months)

   1) Once a week 2) twice a week 3) thrice a week 4)>3 days a week

   5) Once a day 6) twice a day 7) thrice a day

8) HIV status: 1)positive 2) negative 3)unknown

9) Pregnancy:

10) Diabetes Mellitus : Yes/No HbA1c:

Final patient category **A / B / C**

Proforma for ATT induced hepatitis

Case no. **Name** **Hospital No.** **Unique no.**

Contact No **Age** **Gender** 1.Male 2.Female

Place **Basis of Diagnosis**: C / R / M / H / L

Site of TB

1)Sput +ve Pul 2)Sputneg pulm 3)Ple effus 4) LN 5)TBM 6)TB peritonitis 7) Diss 8)TBspine 9)Miliary TB 10)GUTB 11) CNS TB(tuberculomas) 12) TB OM 13) synovitis 14)other

Extent of TB 1.Local 2.disseminated

Current Regimen

Alternate Regimen

Cohort/Presentation 1.Cohort 2.Presentation

Date on initiation of ATT

DILI Date **Time period after initiation of treatment (days)**:

Normalization of LFT (days):

Symptoms

1.Fever 2.Vomiting 3.Abdominal pain 4.Jaundice 5.Rash 6.Others

Signs

1.Jaundice 2.Hepatomegaly(yes/no) 3.Signs of hepatic decompensation 4.Rash 5.Others

Signs of decompensation: 1) Nil 2)Ascites 3)Encephalopathy 4) bleeding

Treatment regular 1.Yes 2.No

Baseline TB DB SGOT SGPT TP Alb Alk phos

Maximum TB DB SGOT SGPT TP Alb Alk phos

Time duration for normalization of LFT (days):

ATT restarted on:

Drug	Date	Dose	Successful

Rechallenge method 1. One drug full dose 2. One drug escalating 3. All drugs

Outcome of Hepatitis:

Severity of hepatitis 1. Mild 2.Moderate 3.Severe 4.Others

Type of hepatitis:

1. Anicteric hepatitis 2.Icteric hepatitis 3.Cholestatic 4.Decompensation

Requirement of hospitalizations 1.Yes 2.No

ICU care 1. Yes 2.No

Severity of hepatitis:

a)complete resolution b)acute fulminant hepatitis c)chronic hepatitis d)Fatal

All cause mortality at completion of treatment 1.yes 2.no 3.Unknown

Number of first line drugs successfully reintroduced:

Drugs successfully rechallenged Rechallenge hepatitis 1. INH 2. RIF 3.PYZ

Outcome of tuberculosis

Treatment completed - 1.Yes 2. No 3. Under treatment 4.Lost follow up

Telephonic follow up 1. Not contactable 2. Treatment completed 3. Defaulted

Cured of tuberculosis 1. Yes 2. No 3.Improved 4.Expired

Duration of treatment (months) Final rechallenge regimen used